DOES DEHYDRATION AFFECT BRAIN STRUCTURE, FUNCTION, AND COGNITIVE-MOTOR PERFORMANCE?

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DOES DEHYDRATION AFFECT BRAIN STRUCTURE, FUNCTION, AND COGNITIVE-MOTOR PERFORMANCE?

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LIST OF SYMBOLS AND ABBREVIATIONS

ATP	Adenosine Triphosphate
AVP	Arginine Vasopressin
BM	Body Mass
BML	Body Mass Loss
BOLD	Blood-Oxygen Level Dependent Responses
CNS	Central Nervous System
CON	Resting Control
CNV	Contingent Negative Variation
CSF	Cerebrospinal Fluid
DCM	Dynamic Causal Modeling
DEH	Dehydration
EEG	Electroencephalography
EHS	Exercise-Heat Stress with Water Replacement
EHS-DEH	Exercise-Heat Stress without Water Replacement
EPSP	Excitatory Post-Synaptic Potential
ES	Effect Size
fMRI	Functional Magnetic Resonance Imaging
FR	Fluid Restriction
MRI	Magnetic Resonance Imaging
NASA-TLX	NASA Task Load Index Scale
ND	No Difference
OVLT	Organum Vasculosum Laminae Terminalis
PCRT	Probabilistic Choice Reaction Task
VEP	Visual Evoked Potentials
VMPT	Visuomotor Pacing Task

SUMMARY

Water comprises the greatest component of body mass. Homeostasis of fluid balance is critical to normal human function since dehydration can result in adverse physiological and medical consequences. Dehydration is also believed to impair cognitive-motor performance, although this has not been consistently demonstrated. Furthermore, if and how dehydration alters specific cognitive-motor domains (e.g., executive function, information processing, memory, reaction time), the visuomotor system (motor planning, motor execution), and sensory systems (e.g., vision, auditory) is poorly understood. In addition, there is a paucity of data regarding the impact of dehydration on brain morphology and function as evaluated by either magnetic resonance imaging (MRI) or electroencephalography (EEG). Therefore, a comprehensive research effort was initiated to determine if dehydration impairs cognitive-motor performance, and, if so: i) which domains are most vulnerable and ii) are performance decrements associated with changes in brain morphology and function.

In Aim 1, a systematic review of the literature combined with meta-analysis of study outcomes and design factors examined the impact of dehydration on cognitive-motor performance. Overall, I found dehydration elicits a small, but significant cognitive-motor impairment. Furthermore, executive functions were the cognitive-motor domain most likely affected by dehydration; whereas, information processing and reaction time were not. There was insufficient data to determine whether dehydration impaired visuomotor performance despite most cognitive-motor tasks in the meta-analysis utilizing visual stimuli. An observation from the meta-analysis was that studies examining visuomotor performance utilized short test durations ($<$ 5 min) and therefore minimized the importance of attention (an executive function). In addition, the short duration studies generated a limited number of observations, minimizing statistical power. Thus, it was determined that: 1) a cognitive-motor test needed to be employed that optimized the ability to discriminate impaired performance from dehydration and provided insight into the affected domains; 2)

an experimental design was needed that induced sufficient body water deficits ($> 2\%$ body mass loss) to induce compensatory physiological responses to maintain fluid balance; and, 3) examining the impact of dehydration on brain morphology and activation concomitant with cognitive performance testing was needed.

Aim 2 determined the additive effects of prior exercise-heat stress and dehydration on visuomotor performance, brain morphology, and brain activation. A well-accepted test of sustained (20 min) visuomotor performance (e.g., visually paced finger tapping) was selected to evaluate motor execution, since only one stimulus-response combination was possible. In addition, this study was the first to simultaneously assess brain activation using fMRI while performing a visuomotor task at body water deficits (3% body mass loss) that require homeostatic responses. This study made the following novel observations: 1) visuomotor performance is impaired with exercise-heat stress and further exacerbated when combined with dehydration; 2) several brain structure volumes are sensitive to both increases and modest decreases in plasma osmolality (as a result of sweat loss or water replacement, respectively); and, 3) additional neural activity was observed following dehydration during a simple finger tapping task. The visuomotor impairments following dehydration in Aim 2 were predominantly attributed to motor execution deficiencies despite eliciting greater brain activation during the task along with changes in anatomical structures in motor areas (e.g., thalamus, basal ganglia). Given that movements require both planning and execution with a greater emphasis on the latter in this Aim, I determined that the effects of dehydration on motor planning required further investigation. Thus, a challenge for Aim 3 was to customize a visuomotor test that sufficiently emphasized motor planning with similar execution demands (i.e., prolonged rhythmic button pressing) to determine if dehydration preferentially impacted either of these components.

Aim 3 examined the impact of dehydration on motor planning as evaluated by performance and brain activations during a visuomotor task. I designed and built a bimanual visuomotor task (probabilistic choice reaction time task; PCRT) with multiple stimuli and

altered presentation frequency (dominant, non-dominant sides) to examine the specific effects of motor planning, motor execution, and attention. PCRT performance (reaction time and accuracy) was evaluated concurrent with function by visual evoked potentials relevant to visuomotor function (Early/Late CNV, N1, N2) using EEG. This study made the following novel observations: 1) performance on a task emphasizing motor planning is impaired with exercise-heat stress coupled with dehydration but not with prior exercise-heat stress alone; 2) motor planning impairments following dehydration may result from increased visual and spatial attentional demands; and 3) dehydration elevated perceived mental workload during a task requiring motor planning. These observations indicate dehydration may impair visuomotor performance, specifically for tasks requiring sustained repetitive movements and vigilance, key elements for ensuring occupational safety. In combination with Aim 2, these data identified that two components of visuomotor function, motor execution and motor planning, are impacted by body water deficits.

This dissertation identified that dehydration impairs the visuomotor system (both motor planning and motor execution) which may explain the observed degradations in human-system interactions with body water deficits. Another common thread through all aims is the contribution of attentional capacity to cognitive-motor and visuomotor decline following dehydration. This is meaningful, as the ability to stay vigilant during monotonous, repetitive eye-hand motor tasks is a large contributor to workplace incidents and driving accidents. Another important observation from Aims 2 and 3 was that some individuals are at greater risk for degraded visuomotor performance following dehydration whereas others sustained their performance. The specific neural mechanisms (structural and functional changes) responsible for these visuomotor degradations (or resilience in some individuals) were not resolved. Further investigations should determine the effects of different forms of dehydration on cognitive-motor performance, the combination of dehydration with other stressors (e.g., sleep deprivation) on cognitive-motor performance, and nutritional countermeasures following dehydration to alleviate cognitive-motor impairments.

CHAPTER 1
INTRODUCTION AND BACKGROUND

There are reasons to believe body water deficits (dehydration; DEH) can adversely impact central nervous system (CNS) function. Severe levels of DEH (e.g., prolonged water restriction in the desert) elicit symptoms of CNS dysfunction such as decreased mental alertness, personality changes (e.g., hostility), and headaches [1, 2]. However, the minimum threshold of DEH which impairs CNS function and whether this level occurs during typical daily living (e.g., $\leq$5% body mass (BM) loss) is less clear. For example, certain populations (e.g., elderly) may be at greater risk for confusion and delirium with DEH, leading to increased frequency, duration, and severity of hospitalizations [3, 4]. Furthermore, small to moderate levels of DEH ($<$ 2% BM loss) may also degrade human-system performance reliant on adequate CNS function, as evidenced by impaired driving ability [5] and performance during occupation-specific simulations (e.g., pilot simulation) [6]. Indirect evidence also suggests occupational accident rates are increased during hot weather/summer months when DEH is likely [7, 8]. Some researchers have suggested drinking water following marginal ($<$ 1% BM loss) can improve memory in both school children and adults [9, 10, 11, 12]. Despite these numerous observations, the peer-reviewed literature remains inconsistent regarding whether moderate DEH (2-4% BM loss) impairs the CNS [13, 14, 15], and specifically, which CNS functions are most susceptible to DEH.

1.1 Physical Performance Following Dehydration

The effects of DEH on physical performance has been extensively reviewed [16, 17, 18]. DEH of $\geq$2% body mass (BM) loss appears to be the threshold eliciting aerobic performance impairments [16, 17, 18] although degradations have been reported at $\sim$1% BM loss [19]. Furthermore, aerobic exercise performance (as measured by time trial or time

to exhaustion tests) appears to be more significantly degraded in warm/hot environments [20, 21, 22] during DEH due to exacerbated cardiovascular strain [23, 22] and high skin temperature as well as other mechanisms related to hyperthermia-induced fatigue [24]. Using skin temperature as an index for skin blood flow requirements, DEH does not appear to impair aerobic exercise performance until skin temperature exceeds $\sim 27^{o}$C, with performance impairments of 1.3% occurring with each 1^{o}C increase thereafter [25, 26]. At lower skin temperatures (at least for the studied exercise intensities), dehydration did not consistently impair aerobic performance.

While aerobic exercise performance is impaired by DEH, the impact of body water deficits on muscular strength is less convincing. A recent review found DEH impaired muscular power in 19% (25/133) of studies eliciting $\geq$4% BM loss [18]. A subsequent meta-analysis [27] indicated DEH significant impairs measures of muscular strength but not anaerobic capacity/power. Experimental evidence has also observed greater performance impairments in muscular endurance (e.g., submaximal contraction time to fatigue) [28], which likely does not result from changes to muscle metabolism [29]. Lastly, DEH may also impair intermittent exercise, as observed with decreases to physical performance in team sports (e.g., soccer) [30]. Thus, the preponderance of evidence appears to indicate that fluid balance is important to aerobic exercise while the effects on both muscular strength and intermittent exercise is less clear.

1.2 Physiological Responses to Dehydration

Dehydration ($\geq$ 2% BM loss) activates physiological mechanisms to maintain homeostasis of water balance [16]. In the body, water is stored within two compartments: extracellular fluid (ECF; 33%) and intracellular fluid (67% of total body water; Figure 1.1) [31], with transfer among these compartments largely governed by osmotic exchange. Within the ECF, fluid exchange between vascular space (plasma volume) and the interstitial fluid is governed by Starling forces (hydrostatic and oncotic) at the capillary. The mechanism

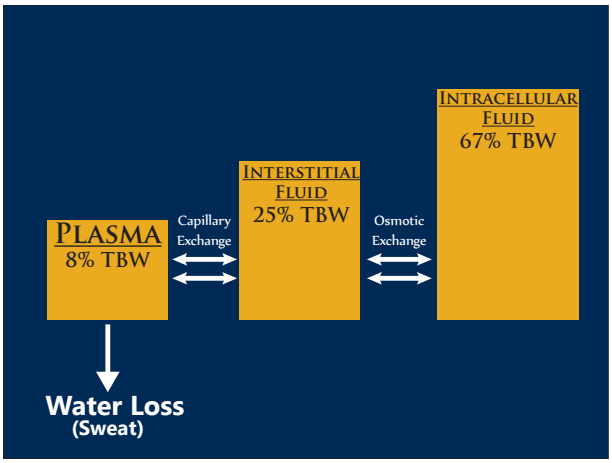

Figure 1.1: Distribution of body water among compartments. TBW = Total Body Water. *Adapted from [31].*

of fluid loss (e.g., sweating, diarrhea, vomiting) influences the osmotic gradient and subsequently the location of fluid loss (intracellular or extracellular) [18]. Isotonic fluid loss (e.g., vomit, secretory diarrhea, medications, high altitude and cold environments) will not alter the osmotic gradient between fluid compartments and is therefore termed isotonic hypovolemia [18]. Conversely, intracellular fluid loss (e.g., water deprivation, sweat, osmotic diarrhea) elicits a hyperosmotic concentration of blood plasma, resulting in fluids being osmotically drawn from the ICF so that a smaller plasma volume reduction occurs for a given water deficit [32]. The mechanism will be discussed in further detail below.

Body water losses elicited by exercise-heat stress (EHS) results in intracellular dehydration. During EHS, elevations in sweat rate (a byproduct of increased metabolic heat production from the exercising muscles) [33] results in marked losses of sweat (i.e., hypovolemia), which is hypotonic relative to blood plasma (i.e., less solute for given volume). Therefore, relatively more water is lost compared to solute resulting in a hypertonic blood plasma (e.g., hypertonic hypovolemia) as evidenced by an increased plasma osmolality. Because cellular membranes are permeable to water and osmotic gradients facilitate movement from high to low concentration, water moves from the interstitial/intracellular compartments into the blood plasma [18, 34, 35]. When extracellular fluid osmolality increases

by $\sim$2%, CNS osmoreceptors for anti-diuretic hormone and angiotensin II and thirst depolarize [36], resulting in water conservation and drive for water acquisition [37].

1.3 Dehydration and Brain Morphology

The effect of DEH on mammalian brain morphology has been of interest for the last 160 years. Early research (before 1930) using simple autopsy measurements of animal organ weight/volume changes revealed that, compared to animals who maintained their hydration status, muscle and skin weights were decreased following severe DEH (multiple days without water) [38, 39, 40] but brain mass was not necessarily affected [39]. More recently, one study in rats corroborated these early findings by observing that, although organ water content decreased following total body water losses of $\sim$16%, total brain water was not decreased [41]. Combined, these studies led to the prevailing belief that brain volume was preserved even with severe DEH, potentially through specialized protective mechanisms.

Until the late twentieth century (after 1990), the ability to image and analyze the *in vivo* human brain was not available. As a result, the first usage of neuroimaging to scan the human brain following DEH occurred in 2005 [42, 43]. The first two studies reported different results, with one observing no differences in brain volume following DEH of -2.2% BM [42], but the other finding a significant decrease in total brain volume at -1.6% BM [43] (Table 1.1). To date, eight studies have investigated the effects of DEH on brain morphology (Table 1.1), with most reporting on total brain volume and/or lateral ventricle size. The only report of decreased total brain volume (of $\sim$0.5%) is from the aforementioned study which induced 1.6 % BM loss using a 16-h fluid restriction protocol and had individual responses ranging from +0.2 to -2.4% [43]. The authors hypothesized decreased brain volume resulted from the osmotic gradient induced by hypertonic hypovolemia shrinking the astrocytes which minimized fluid movement within the brain [43]. However, this study is at odds with other studies reporting no change in total brain volume with DEH ranging from 0.8 - 2.9 % body mass (BM) loss [44, 45, 46, 47].

Table 1.1: Previous Studies Using MRI to Investigate Dehydration (DEH) on Brain Morphology. EHS = Exercise Heat Stress, ND = No Difference (from control), CSF = cerebrospinal fluid, FR = Fluid Restriction. * Different versus a hyperhydration condition.

Citation	**Subject n**	**Method of DEH**	**$\triangle$ BM**	**Findings**
Total Brain Volume				
Duning et al. 2005 [43]	10 (3 M)	FR	-1.6%	$\downarrow$ Brain Volume
Dickson et al. 2005 [42]	6 (6 M)	EHS	-2.3%	ND
Kempton et al. 2009 [45]	7 (7 M)	EHS	-2.2%	ND
Watson et al. 2010 [44]	8 (8 M)	EHS	-2.9%	ND
Nakamura et al. 2014 [48]	14 (12 M)	FR (2wk prior)	?	ND
Meyers et al. 2016 [47]	20 (11 M)	FR	-0.8%	ND
Brain Ventricles				
Dickson et al. 2005 [42]	6 (6 M)	EHS	-2.3%	ND
Kempton et al. 2009 [45]	7 (7 M)	EHS	-2.2%	$\uparrow$ Lateral Ventricle Volume
Watson et al. 2010 [44]	8 (8 M)	EHS	-2.9%	$\downarrow$ Total Ventricle & CSF Volumes
Kempton et al. 2011 [46]	10 (5 M)	EHS	-1.6%	$\uparrow$ Lateral Ventricle Volumes
Streitburger et al. 2012 [49]	6 (3 M)	FR	-2.3%	ND
Meyers et al. 2016 [47]	20 (11 M)	FR	-0.8%	ND
Grey and White Matter				
Streitburger et al. 2012 [49]	6 (3 M)	FR	-2.3%	$\downarrow$ Grey Matter*, $\downarrow$ White Matter*
Meyers et al. 2016 [47]	20 (11 M)	FR	-0.8%	ND

Six studies have attempted to determine changes within the lateral ventricles following DEH, as these structures are both the largest component of the ventricular system (i.e., repository for brain water) and a prominent/distinguishable landmark when imaging the brain. However, unlike total brain volume, these data are more divergent. While some studies have observed no significant difference [47, 42, 49, 47, 42], others have reported either an increase [46, 45] or decrease [44] in lateral ventricular volume following DEH ranging from 0.8% to 2.9% BM loss. Furthermore, two studies have suggested that lateral ventricle volume expansion significantly correlates to BM loss [46, 42], such that the magnitude of body water loss elicits a graded increase in lateral ventricular volume. However, in both studies, these correlations were observed over a small range of DEH ($\sim$1%), a wide range of ventricular volume changes ($>$14%), and the sample included subjects with ventricular expansion and shrinkage.

While multiple studies have presented data regarding potential brain ventricle volume changes following DEH, the impact of body water deficits on other brain structures has received little attention. Following DEH of 2.3% BM loss, Streitburger et al. [49] observed decreased white and grey matter volumes in areas surrounding the brain ventricles when compared to when subjects hyperhydrated (i.e., consumed water in excess). Although these data are interesting, the small sample size (n = 6), little control for baseline hydration status, and comparing between DEH with hyperhydration (and not euhydration) make their interpretation less convincing. Furthermore, the voxel-based morphology approach did not allow for individual brain structure segmentation, and therefore additional information about brain areas possibly susceptible to DEH cannot be determined. Therefore, a goal of this thesis is to improve previous limitations in research design (i.e., establish a clear baseline threshold to ensure euhydration, examining a level of DEH observed to cause homeostatic mechanisms to maintain fluid balance [50]) and delineate the comprehensive impact of DEH on brain structures specific to motor functions.

1.4 Water Homeostasis in the Brain

Given the heterogeneity in observed changes to brain ventricles, questions remain regarding a potential mechanism by which DEH may expand/contract the ventricular system. Whole body osmoregulation occurs via activation of both peripheral (e.g., within the digestive tract) and central osmoreceptors (e.g., those within the brain) [36]. The primary central osmoreceptors are located in brain areas without presence of a blood-brain barrier, the circumventricular organs, specifically the *organum vasculosum laminae terminalis* (OVLT), located at the base of the third ventricle [36]. As the OVLT is exposed to hypertonic fluid, the cellular volume shrinks (intracellular DEH), leading to a depolarized state of transient receptor potential vanilloid 1 receptors [51] and increased frequency of action potentials [36]. Secondly, although not strictly osmoreceptors, many cellular types show osmoreceptive properties. Glial cells, specifically astrocytes, regulate brain water and ion homeostasis in addition to maintaining the blood brain barrier [52]. Following the sensing of hypertonicity within the blood, arginine vasopressin (AVP) is released both systemically (to the body) and centrally (to the brain) from the posterior pituitary [52]. AVP activates the uptake of ions (Na^+, K^+, Cl^-, Ca^{2+}) within astrocytes to accomplish two things: i) influx of Na^+, K^+, and Cl^-, ii) over-expression of aquaporin 4 to allow influx of water [52].

Within the brain, the choroid plexuses secrete $\sim$80% of the cerebrospinal fluid (CSF) [53, 54]. The choroid plexuses lie within each ventricle (two within the lateral ventricles) in close proximity to the ventricular cavity [54], creating the blood-CSF barrier. The choroid plexuses receive an abundance of blood flow from various cerebral arteries, $\sim$10x greater than the rest of the brain [54]. CSF secretion from the choroid plexuses occurs from i) ion pumps located within the plexus epithelium which facilitate ion movement from blood into the CSF (in a ratio of 18 Na+, 15 Cl-, 3 HCO3-) and ii) aquaporin channels allowing water flow from blood to CSF [54]. It appears that CSF is isotonic to blood plasma [55]. Therefore, changes to the blood plasma tonicity may also be reflected in the CSF. In hy-

pernatremic rats, 30 min of hypernatremia decreased CSF secretion and minimized Na^+ uptake [56]. Thus, in hypertonic hypovolemia, increased tonicity of blood plasma may slow CSF production, potentially shifting water out of the brain tissue to equilibrate the osmotic gradient.

In addition to the blood-CSF barrier, osmoprotective mechanisms (e.g., ion pumps, leak channels) exist within the brain to maintain total brain volume despite large body water losses [41, 53, 57]. The brain, unlike other body tissues, does not allow water influx by Starling forces, as the presence of high electrical resistance tight junctions ($\sim$100x greater than capillaries) within the blood brain barrier (BBB) prevent movement of electrolytes and other hydrophilic substances [58, 53, 59, 60]. These protective mechanisms are also bolstered by astrocytes, which assist in maintaining structural integrity of the tight junctions, regulating ionic homeostasis, and buffering K^+ [53]. Secondly, the brain produces organic osmolytes (myo-inositol, betaine, taurine, sorbitol) which help preserve cellular volume by increasing ionic concentration within the cell, equilibrating the osmotic gradient with the hypertonic exterior [61, 62]. During chronic (7 d) hypertonicity (extracellular osmolality greater than intracellular osmolality), organic osmolytes accounted for 35% of brain tonicity (with no change in brain water content) [63] and were up-regulated by 44% in an infant suffering severe DEH [64]. However, because organic osmolyte gene encoding occurs following sensing of hypertonicity, appearance within the brain tissue is delayed [61, 65]. As a result, osmolyte-mediated equilibration of neuronal water content does not occur immediately following acute exposure to hypertonic environment [66]. Therefore, although humans may be protected against large brain water perturbations, the delayed time course of osmolyte production may result in vulnerabilities to brain water homeostasis during acute DEH as elicited by exercise-heat stress.

1.5 Cognitive-Motor Responses to Dehydration

It has been well appreciated that, under environmental extremes and conditions eliciting marked dehydration, severe psychological consequences are evident [1, 2, 67]. However, the more pertinent question remains if impairments in cognitive-motor performance occur at DEH levels frequently encountered by those in military, occupational, or athletic environments [17]. Four early studies were seemingly the first to examine the specific effects of DEH on cognitive performance, observing mixed results [68, 69, 70, 71]. Three of these studies reported cognitive-motor domains of executive function and information processing were impaired at moderate levels of DEH (-2% BM) with further deterioration as the magnitude of DEH became greater [71], particularly in hot conditions [69, 70].

Following these early studies, various levels of DEH and methods to induce DEH have been utilized across a number of investigations without being able to replicate these large deteriorations in cognitive-motor performance. While most review articles suggest variability in study design largely explains the disparate literature [72, 73], studies controlling for some of these variables have not always supported this claim. For example, when the magnitude of DEH was controlled (2.8%), DEH induced by both exercise heat stress and heat stress alone deteriorated short term memory and perceptive discrimination [74]. This study was followed up a year later [75], replicating findings of impaired information processing and memory, with additional perceptual measures revealing DEH (by both exercise and heat stress) elevated fatigue during the cognitive-motor battery.

Cognitive-motor task/domain selection may be a larger contributor to the wide variation within the previous literature [13, 14, 76, 77]. Most previous studies employed a multimodal battery assessing a wide array of cognitive-motor domains (e.g., executive function, working memory, reaction time, information processing). As a result, there are commonly observed within-study discrepancies, with some aspects of cognitive-motor function being impaired while others remain unaffected [76, 77, 75]. One hypothesis is that DEH may

more severely impair the higher-order cognitive-motor functions (e.g., executive functions) compared to simpler tasks (e.g., simple reaction time) [77]. Evidence for this has been observed previously [77], with more difficult variations of an executive function task eliciting greater errors than the less complex variation following DEH. However, based on other studies, this conclusion lacks consistent support in the literature [78, 15]. Executive functions (e.g., working memory, executive function specific tests, inhibitory control) are not been uniformly impaired across all studies [79, 80, 15, 81, 82], although some studies only elicited modest levels of DEH ($<$2% BM loss). Furthermore, some studies have also observed decrements in lower level tasks, such as simple reaction time [83, 84], indicating cognitive-motor performance impairments may not be isolated to "complex" tasks.

Recently, three [85, 5, 46] investigations have attempted to better understand how DEH may impair brain function by employing neuroimaging techniques while performing cognitive-motor tasks. The first such investigation [85] utilized electroencephalography (EEG) and assessed auditory event-related potentials (P3) with an oddball paradigm. Measuring three central electrodes on the frontal, central, and parietal regions, there was no impact of DEH (2.6% BM loss via 24 h fluid restriction) on either latency or amplitude of the auditory event-related potentials [85]. However, despite no changes in EEG responses, subjects did report increased levels of effort and concentration required to complete the cognitive-motor tasks following DEH, suggesting the tasks were more strenuous although not to the extent of impairing function or performance [85]. Whether responses are similar depending on the method that DEH is induced (e.g., exercise and/or heat stress), as has been previously hypothesized [86], is not currently known.

Following this initial study, two others [46, 87] have employed functional magnetic resonance imaging (fMRI) to examine the effects of DEH on cognitive-motor performance and function. fMRI has the benefit of added spatial resolution along with the ability to simultaneously investigate brain activations within cortical, subcortical, and cerebellar brain areas [88] while performing cognitive-motor tasks. One study found elevated fronto-parietal

blood oxygen level dependent (BOLD) responses during an executive function task (Tower of London) following DEH (1.6% BM loss) [46]. However, DEH did not alter executive-function task performance [46], and therefore, the authors suggested elevated BOLD following DEH indicates inefficient neural processing that may not necessarily be reflected in behavioral impairments. More recently, another EEG study [5] suggested alpha (8-11 Hz) and theta (4-7 Hz) power within the motor brain regions (C3, C4 electrodes) may be increased following marginal DEH (1.1% BM loss) during a prolonged, monotonous, driving task. This was coupled with an increase in errors following DEH, suggesting that, over an extended (120 min) period, DEH may increase the neural resource requirement leading to impaired performance. However, given the analysis within the previous study [5], it is difficult to elucidate whether increased brain activation resulted from increased effort, task-related impairments, or another potential behavioral explanation. Lastly, one study has examined the effects of DEH on pain-evoked-activations (cold presser test) with fMRI [87]. DEH elicited greater BOLD responses within the anterior cingulate, insula, and thalamus compared to controlled conditions along with a decreased pain threshold, suggesting somatosensory function may also be altered. Taken together, these initial neuroimaging studies have begun to suggest DEH may alter neural resource requirements/activation patterns during cognitive-motor or sensory tasks as a mechanism by which body water deficits affects cognitive-motor function and performance.

1.6 Dehydration and Visuomotor Function

Studies assessing the effects of DEH on cognitive-motor performance typically employ computerized tasks requiring subjects to produce a movement (e.g., button press) which activates the visuomotor system. While the primary and supplementary motor cortices were once believed to govern motor movement, recent evidence suggests these areas also respond to the presentation of visual stimuli within the frontal eye field [89]. The entire visuomotor system consists of a multitude of fronto-parietal brain areas (prefontal cortex,

supplementary eye field, frontal eye field, primary motor cortex), subcortical structures (thalamus, basal ganglia, hippocampus), and the cerebellum which combine to encode a motor program to facilitate completion of an action (e.g., pressing of button) [90]. This thesis will further explore visuomotor function using visually paced finger tapping tasks. Within these visually paced tasks (e.g., responding to a blinking light), researchers have primarily been interested in the timing mechanisms and potential influences [91]. The tasks can take many variations including the number of fingers required, nature of stimulus (visual or auditory), and whether the pacing is external (e.g., stimulus on screen) or internal (e.g., tapping at an internally-driven rhythm) [92]. Furthermore, performance effects can also widely vary based on the frequency of tapping [93].

Broadly, the production of a motor response occurs in two phases: motor planning and motor execution [94, 95]. Motor planning consists of the cognitive steps involved before movement occurs: observing the environment (i.e., stimulus identification), integrating task rules (i.e., if stimulus is red, press right button), determining the motor goal (i.e., press right button), and the abstract kinematics (i.e., how to move right) [96]. It is believed motor planning, because of the information processing involved, dictates the measured reaction time within visuomotor studies [96]. Although motor planning and execution are presented here as occurring serially, some have suggested neurons within the primary motor cortex and dorsal premotor cortex may contribute simultaneous to both motor planning and motor execution [94].

Motor responses occur via integration of the motor thalamus, basal ganglia (globus pallidus internus, substantia nigra pars reticulata), cerebellum, and the associative, premotor, and motor areas of the cerebral cortex (Figure 1.2) [97, 98, 99]. Within motor execution, it appears that the motor thalamus is integral to integrating various pieces of information (e.g., motor program, temporal pattern, motivation, action selection) to select/execute the correct motor movement [98]. However, the thalamus also has a well established sensory role with integrations from the visual cortex along with mediating arousal, attention, and

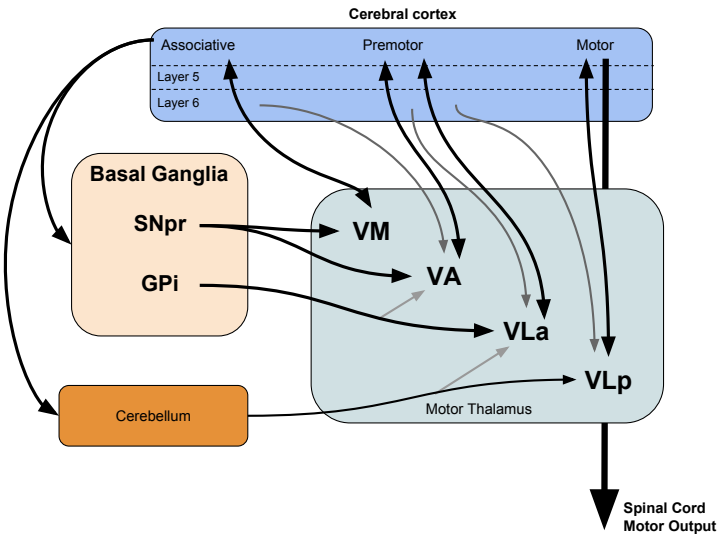

Figure 1.2: Connections within the thalamus during motor execution. SNPi = substantia nigra pars reticularis, GPi = globus pallidus internus. Thalamusic nuclei: ventral anterior (VA), ventral later anterior (VLa), ventral lateral posterior (VLp), ventral media (VM). *Adapted from [98].*

other sensory stimuli [100]. Because DEH degrades perceptual responses involving sensory mechanisms (e.g., mood, thirst, hostility) [101, 80, 67], the thalamus, and thus visuomotor function, could be uniquely challenged by body water deficits.

Despite the potential risk for visuomotor impairments by thalamus overload (or other neurophysiological mechanism), previous research has not systematically investigated the effects of DEH on motor planning and motor execution. Furthermore, no study has previously examined how visuomotor brain areas (motor cortex, supplementary motor cortex, basal ganglia, thalamus, cerebellum, parietal cortex) are impacted by DEH. Previous research has examined some components of visuomotor function (Table 1.2), however, the results are not clear. One example of this discrepancy in the literature occurred in two studies from the same lab [75, 74]. Using an identical DEH protocol, $\sim$3% BM loss, and unstable tracking task, one study reported impaired motor coordination [74] while a subsequent study did not [75]. Despite the equivocal literature, reports of slowed visuomotor reaction time [102] and decreased motor coordination accuracy [70, 74] have been observed

Table 1.2: Studies utilizing aspects of visuomotor functioning or occupation specific task following dehydration. BM = Body Mass.

Study	% BM Loss	Task	Self-Reported Findings
Sharma et al. [70]	1-3	Psychomotor Stylus Test	Longer Completion Time at $\geq$2% BM loss
Patel et al. [105]	2.5	Balance Error Scoring System	—
Cian et al. [74]	2.8	Unstable Tracking Task	Increased Deviation ($p < 0.05$)
Cian et al. [75]	2.8	Unstable Tracking Task	—
Wong et al. [102]	1.4 - 2	Psychomotor Reaction Time Test	Decreased Speed ($p < 0.05$)

following DEH, suggesting both motor planning and motor execution may be impaired. Changes within visuomotor task performance may manifest/contribute to deteriorations in skilled task performance, which has been previously observed [103, 104, 30].

1.7 Utilizing Neuroimaging Techniques to Assess Visuomotor Function

Employing neuroimaging techniques which allow for the examination of how DEH may alter brain activation levels/patterns is essential to further understanding of how body water deficits may impact cognitive-motor functioning. This thesis will utilize both functional magnetic resonance imaging (fMRI) and electroencephalography (EEG) following controlled DEH induced by exercise-heat stress. Neuroimaging techniques are characterized by their temporal and spatial characteristics, with EEG allowing for greater temporal resolution (order of milliseconds) than fMRI (order of seconds) while fMRI has the benefit of greater spatial resolution (5 vs 20 mm, respectively; Figure 1.3) [88]. The relative strength of each neuroimaging modality is derived from the signal measured, as described below.

Magnetic Resonance Imaging

Magnetic resonance imagining (MRI) utilizes magnetic fields to compute a three-dimensional (four including time) image of the human brain with precise (1 mm^3) resolution [106]. When an individual is placed within the MRI scanner, the static magnetic field (B_0; strength measured in Tesla) causes the spin angular momentum, or 'spin', of hydrogen protons (located within water molecules), to align their magnetic moments either parallel or anti-

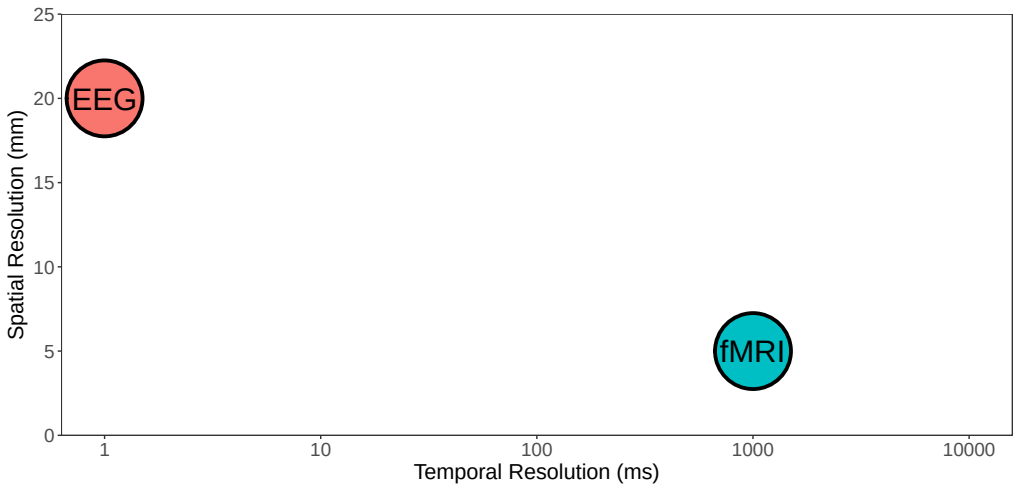

Figure 1.3: Comparison of the spatial and temporal resolution of electroencephelography (EEG) and functional magnetic resonance imaging (fMRI).

parallel to B_0, creating some degree of field homogeneity. When a radiofrequency pulse (at the Larmor frequency) is applied, a percentage of the protons will become excited, creating resonance within the magnetic field [106]. This resonance (and subsequent relaxation of the protons) is measured using a receiving coil and forms the basis for image reconstitution.

Different molecular composition within the brain alters the level of resonance/relaxation allowing for differentiation between tissue types. Furthermore, by manipulating scan parameters, the differentiation between tissue types can be highlighted for optimal clarity. For example, when the scan is weighted towards T2 (transverse plane) relaxation, the cerebrospinal fluid will be much brighter than with a T1 (longitudinal relaxation) scan [107]. The echo time (TE; between radiofrequency pulse and peak of magnetic resonance signal induced in the receiver coil) and repetition time (TR; time between subsequent radiofrequency pulses) of a scan will dictate which tissues are most discernible from one another [108]. For example, a classic anatomical scan (T1) utilizes a short TE and short TR which allows fat (within myelin sheaths; quick recovery) to appear brighter than water (grey matter; long recovery).

By utilizing these core aspects of MRI, it is possible to measure brain activity. Func-

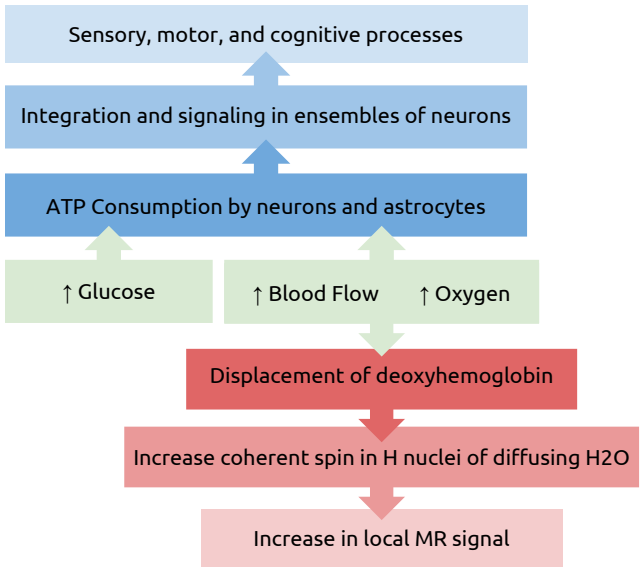

Figure 1.4: Metabolic processing leading to measurement of the magnetic resonance signal. ATP = Adenosine Triphosphate.

tional MRI (fMRI) originates from the dynamic blood flow within the brain during cognitive-motor processing (Figure 1.4). Specifically, fMRI utilizes the magnetic properties of blood [109] as the indicator of brain activation. When oxygen is bound to hemoglobin, the blood is diamagnetic (i.e., minimal magnetic properties) whereas blood with deoxyhemoglobin is paramagnetic (i.e., has magnetic properties) [109, 110]. During cognitive-motor processing, the brain disproportionately increases local blood flow in the form of elevated oxyhemoglobin in the draining vein [109, 110]. The blood within the draining vein, consisting of a lower ratio of paramagnetic to diamagnetic blood compared to rest, creates a more homogeneous local field [110, 109]. The more homogeneous local field leads to longer relaxation times, and thus increased signal intensity [109]. The increased signal intensity is termed the blood-oxygen-level dependent (BOLD) response, as the oxyhemoglobin level within the draining vein will dictate the signal intensity.

The BOLD response occurs in four phases. Two seconds after presentation of a single stimulus, there is a small initial decrease in signal change from rest which is believed to be caused by a temporary decrease in oxygenation levels within the capillary [111]. As the

neurons detect an increased oxygen demand, the local vasculature dilates, increasing blood flow and with it greater BOLD responses. After hitting a peak (with potential to overshoot demand), the BOLD response is sustained during the length of processing. Following stimulus presentation, the BOLD response undershoots (i.e., negative relative to baseline) resulting from the increased local blood volume combined with minimal flow, leaving a high oxyhemoglobin concentration within the specific brain region.

The primary weakness of fMRI/BOLD is the doubly-dissociated nature of the signal (compared to direct measurement of neural activity). Previous investigations have determined that the BOLD response reflects increases in neural activity [112]. Specifically, the BOLD response appears to reflect local field potentials, suggesting elevated signal indicates processing within a specific brain region compared to individual neuronal spiking [112]. The advantages of fMRI are the spatial acuity and ability to measure brain activation throughout the entire brain (i.e., cortex, subcortical areas, and cerebellum). However, given the indirect nature of BOLD responses, the temporal resolution is limited to 4-6 s following stimulus presentation [112]. Experimentally, these weaknesses can be minimized by employing a block-design paradigm which utilizes repetitive 'active' and 'rest' periods, allowing for a sustained plateau in BOLD response ($\geq$ 10 s) compared to a single stimulus. The active period signal change is then compared to the rest period to create contrasts indicating regions with greater activation during cognitive-motor processing.

Electroencephalography

In contrast to the dissociated nature of the BOLD response, EEG measures electrical activity of the neurons from surface electrodes versus magnetic fields. These surface electrodes are housed within an elastic cap which allows for stabilization and consistent placement across the scalp. However, although the scalp placement for each electrode can be controlled, cortical neurons vary greatly in their orientation relative to a given surface electrode [108]. As a result, EEG measures post-synaptic cortical fields and not single action

potentials [108, 113, 108].

When excitatory projections synapse onto dendrites, an excitatory post-synaptic potential (EPSP) forms via repeated depolarization. This EPSP results in the intracellular area of the dendrite becoming transiently positive while the extracellular space is negatively charged. The dendrite also becomes a current sink, as the influx of sodium ions facilitates the electronic current traveling down the dendrite to the soma (current source). As current travels down the dendrite, the EPSP will decrease in strength. Therefore, depolarization will be the greatest at the site of innervation and weakest by the cell body, creating a dipole. Over time, the EPSPs from thousands of neighboring (and spatially aligned) neurons will sum and create a measurable signal for the surface electrodes. The surface electrodes are then amplified and compared to a neutral electrode before being fed into a data acquisition system as a measure of brain activation (Figure 1.5).

When EEG measurements are repeated multiple times, a pattern of electrical changes will occur relative to the stimulus onset. These are titled visual evoked potentials (VEP) [113]. The presence of VEP results from neural processing at specific time-periods relative to stimulus onset (e.g., visual object, auditory sound) (Figure 1.5). VEP are named for the duration following stimulus onset and whether the inflection is positive or negative [113, 114, 115]. VEP have clinical relevance [116, 117] and thus can provide valuable insight into cognitive-motor processing. Specifically, when compared to BOLD, the VEP allow for greater temporal specificity (on the order of miliseconds vs. seconds) and allows for investigation into specific stages/phases of cognitive-motor processing.

The visual evoked potentials were first identified in 1964 with the contingent negative variation (CNV) [118]. The CNV is a negative deflection occurring after a warning stimulus (but before cue to move) [113] and appears to be related to processing a warning stimulus and anticipation of an upcoming movement [119]. VEPs occuring after stimulus onset may reflect neural activity related to sensory components (i.e., identification of a stimulus), task-dependent neural processes, or motor components of a task [113]. The N1, by naming

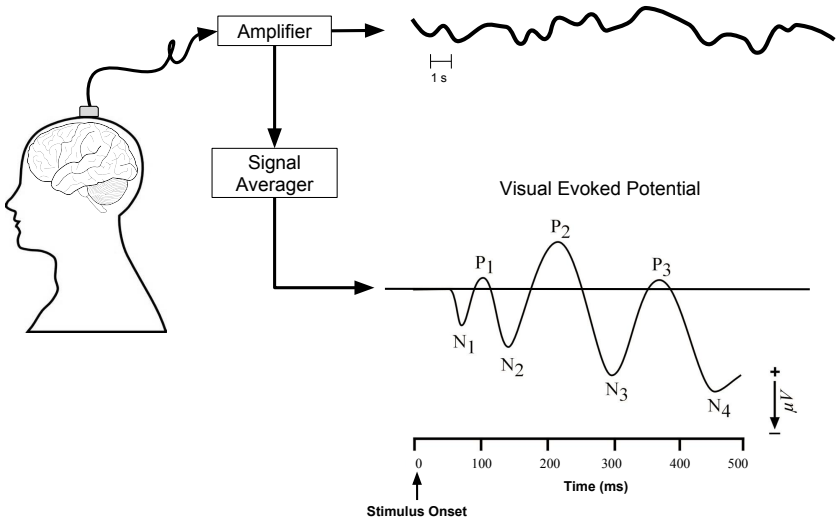

Figure 1.5: EEG signal and processing stages. Visual evoked potentials refer to the inflection (P = positive, N = negative) and time following sitmulus onset (1 = 100 ms, 2 = 200ms, 3 = 300ms, 4 = 400 ms).

convention, is a negative deflection occurring ∼100 ms following stimulus presentation in the posterior electrodes [113] and has been previously observed to be important in motor planning and perceptual processing [120, 121]. The N2, a negative deflection occurring approximetaly 150-180 ms after stimulus presentation represents neural processing relative to stimulus categorization (e.g., frequent, infrequent) [113, 122]. The P2, a positive deflection occurring 200 ms stimulus presentation, is traditionally observed within the anterior and central brain regions [113] and is associated with motor initiation [120]. The P3, a positive deflection occurring ∼300 ms following the onset of a stimulus, is one of the most commonly investigated VEP components [113]. The P3 is associated with stimulus evaluation [114] and occurs frequently within choice reaction time tasks [123] within the central parietal lobe.

1.8 Research Aims

Dehydration is known to impair sustained physical performance in athletic, occupational, and military environments [16], however, the effects on performance of cognitive-motor tasks are less definitive. Cognitive-motor performance is typically assessed with a computerized task where subjects produce a movement (e.g., button press), requiring activation of the areas involved in creating a motor response (i.e., visuomotor system). However, to date, the effect of DEH on visuomotor performance (both motor planning and execution) has not been systematically evaluated. Furthermore, relatively few studies have integrated neuroimaging measures during the performance of cognitive tests following DEH (and none during visuomotor tasks) [46, 5, 85], to examine the impact of body water deficits on brain structures and function. By testing the following specific aims, we will evaluate how DEH impacts performance and neural activity during visuomotor tasks emphasizing motor planning and motor execution along with changes brain morphology.

Research Aim 1: To systematically review the literature and examine factors influencing the effect size of dehydration on cognitive-motor functions via meta-analysis.

Based on conclusions from multiple review articles [72, 86, 73], it is still unclear: i) whether dehydration significantly impairs cognitive-motor performance and ii) under which conditions potential impairments are observed. To assess this, in Chapter 2, a systematic review of literature is presented examining the effects of dehydration on cognitive-motor performance. All cognitive-motor tasks will be classified into specific domains (e.g., executive functions, information processing, memory, reaction time) based upon a previous protocol [124].Additional study design factors will be extracted and statistically analyzed using a meta-analytic approach to examine the effect size of dehydration on different cognitive domains and the level and method of dehydration.

Previous literature has suggested that, if dehydration impairs cognitive-motor performance, the decrements will be small - similar to the effect observed with other nutritional

interventions [72]. Previous literature has also suggested cognitive-motor impairments are associated with the magnitude of DEH, as evidenced by studies observing exacerbated cognitive-motor decrements with increased body water losses [71, 70]. Narrative reviews have also concluded cognitive-motor decrements may increase at larger (3-4%) body water losses [30] and as the task complexity increases (e.g., greater errors during the more complex version of an executive function task) [77, 30].

Research Hypotheses:

- Dehydration will adversely affect cognitive-motor performance
- Cognitive-motor impairments will be significantly associated with the magnitude of body mass loss
- Dehydration will impair higher order cognitive-motor domains (e.g., executive functions) to a greater extent than lower-level domains (e.g., simple reaction time)

Research Aim 2: To examine the effect of exercise-heat stress with and without water replacement on brain structure, function, and visuomotor performance during a task emphasizing motor execution.

Most studies agree total brain volume is unchanged following dehydration (Table 1.1) although the effect of body water deficits on specific brain structures is not clear. Brain ventricles may either increase, decrease, or not change following dehydration while other structures have not been previously studied (Table 1.1). It is plausible that, if plasma becomes hypertonic due to dehydration, osmotic gradients would shift fluid out of brain tissues and into extracellular spaces (i.e., ventricles). While this may explain increased ventricular volume following dehydration [46, 45], the concomitant shrinking of brain structures has not been previously described or demonstrated based on changes in plasma tonicity.

Secondly, in the few fMRI studies available [46, 87], dehydration increases brain activation although cognitive-motor performance may be preserved. However, these findings were applicable only for higher-order cognitive-motor functions [46]. How the visuomotor

system, particularly motor execution, is impacted by DEH has not been throughly examined [75, 74, 70]. Moreover, structure-function effects have not been directly examined. Therefore, the impact of dehydration on brain activation patterns during visuomotor tasks related to brain structural changes merits comprehensive investigation. In Research Aim 2, I will elicit dehydration (following exercise-heat stress) to examine brain structural changes and activation patterns during a fundamental visuomotor task emphasizing motor execution (finger tapping) compared to resting control and a matched exercise-heat stress bout with water replacement.

Research Hypotheses:

- Dehydration will increase blood-oxygen level dependent (BOLD) responses while performing a visuomotor task but not significantly impair performance based on reaction time or accuracy.

- Dehydration (resulting in hypertonic hypovolemia) will not alter total brain volume, but will induce expansion of the brain ventricular system while decreasing volume of the surrounding tissue structures.

- Exercise-heat stress with water replacement will attenuate any brain structural changes and preserve visuomotor performance without requiring greater neural activation.

Research Aim 3: Evaluate the impact of dehydration on performance and function during a visuomotor task emphasizing motor planning.

The visuomotor task in Aim 2 was selected in order to emphasize the motor execution phase of visuomotor function. To investigate motor planning in Aim 3, a bimanual visually-paced task (paced choice reaction task) [125, 96] was evaluated under the same exercise-heat stress test protocol used in Aim 2. A paced choice reaction task will also utilize different weighting schemes (between dominant and non-dominant sides) to bias the motor planning system [125]. Electroencephalography (EEG) during the motor visuomotor task will enable the investigation of neural processes following stimulus presentation

(specifically during motor planning). Analysis will include the contingent negative variation (CNV) [118] and two other visually evoked potentials which occur with serial button presses relevant to visuomotor function - the N1 and N2 [120, 122], specifically within the central and occipital regions. Previously, dehydration did not alter auditory-evoked P3 amplitude during an oddball paradigm [85]; however, VEP responses during a visuomotor task following dehydration have not been investigated. N1 and N2 amplitude appears related to processing demands [113] to visual/spatial attention and stimulus categorization, respectively. VEP amplitude may also be associated with visuomotor task performance [120], providing insights into function-performance relationships. Secondly, during visuomotor tasks, CNV amplitude appears to reflect preparatory processing within the central regions [126] with the amplitude being suppressed during lower task demands [119]. Since previous research suggests dehydration increases perceived mental workload [85], I also will evaluate the effects of dehydration on mental workload (e.g., effort, frustration) of the motor planning task.

Research Hypotheses:

- Dehydration will impair accuracy (but not reaction time) during a visuomotor task emphasizing motor planning.
- Dehydration will alter brain activity by increasing the peak amplitude of the N1 and N2 but decrease CNV amplitude
- Motor planning performance and perceived mental workload will be directly associated with visual evoked potential amplitude.
- Dehydration will increase mental workload compared to both resting control when dehydration is prevented during exercise-heat stress

CHAPTER 2

AIM 1: DOES HYPOHYDRATION IMPAIR COGNITIVE-MOTOR PERFORMANCE: A SYSTEMATIC REVIEW AND META ANALYSIS

2.1 Abstract

Dehydration (DEH) is believed to impair cognitive-motor performance but which domains are primarily affected and at what magnitude of body mass loss (BML) remains unclear. PURPOSE: To conduct a systematic literature review and meta-analysis to determine the effect size (ES) of DEH on cognitive-motor performance and the influence of experimental design factors. METHODS: Thirty-two studies were identified, providing 258 ES estimates from 397 subjects with DEH ranging from 1-6% BML. Outcome variables (accuracy or reaction time), cognitive-motor domains, and methods to induce DEH varied. ES were calculated using standardized mean differences and multivariate meta-analysis. RESULTS: Impairment of cognitive-motor performance (all domains/outcomes) with DEH was small but significant (ES = -0.22; 95% CI: [-0.33, -0.12], $p < 0.0001$) with significant heterogeneity ($Q(257) = 683.8$, $p < 0.0001$; $I^2 = 37.6\%$). Both task accuracy (ES = -0.26; [-0.38, -0.15]; $p < 0.0001$) and reaction time (ES = -0.13; [-0.26, -0.01]; p = 0.04) were significantly impaired following DEH. Cognitive-motor impairment following DEH was greater ($p < 0.001$) for executive functions (ES = -0.28; 95% CI: [-0.39, -0.16]) compared to simple/choice reaction time tests (ES = -0.09; [-0.22, 0.04]). BML was associated with the level of cognitive-motor impairment ($r^2 = 24.9\%$; p = 0.03); consequently, $>2\%$ BML (ES = -0.30, 95% CI: [-0.43, -0.17]; 123 outcomes) had greater impairment (p = 0.02) compared to $\leq 2\%$ (ES = -0.14, 95% CI: [-0.27, -0.00]; 135 outcomes). CONCLUSIONS: Despite variability among studies, DEH significantly impairs cognitive-motor performance, particularly for higher-order tasks involving executive functions when water deficits are above

2% body mass loss.

2.2 Introduction

Dehydration (DEH) has known adverse effects on the human body [17, 16]. It is well-documented that physical performance tasks (aerobic exercise, muscular endurance, occupational tasks, sport-specific tasks) are impaired with DEH [17, 103, 6, 27]. In contrast, which cognitive-motor domains are most susceptible to dehydration and at what threshold of body water loss is far less clearly defined [18]. Initial studies on DEH and cognitive-motor performance suggested executive function and information processing were impaired following DEH of 2% body mass loss (BML) [70, 71]. However, these findings have not been uniformly supported in subsequent studies [14, 13, 127]. No sole reason accounts for the equivocal findings within the literature, however, potential variables include differences across methods to elicit DEH (e.g., exercise, exercise-heat stress, fluid restriction, diuretics), the magnitude of DEH, and the specific cognitive-motor task evaluated [86, 128, 72]. While narrative reviews highlight potential factors influencing the cognitive-motor responses to DEH [73, 128, 86, 72, 30], a quantitative analysis that systematically examines the effect of these variables is absent from the literature.

It is clear that marked levels of DEH (e.g., $>$8% BML) in environmental extremes and harsh conditions elicit severe psychological impairments [2, 1]. Soldiers in adverse environments (e.g., desert heat with extended water restriction) have an impaired ability to navigate, successfully complete military operations, and, if DEH is severe enough, present with confusion and delirium [2, 1]. Soldiers undergoing 5% BML during a 72 h training exercise had impaired (by 2-4 fold) vigilance, reaction time, attention, memory, and reasoning compared to their performance at rest [67]. However, these field-based military studies inducing large magnitudes of DEH typically include other co-factors also known to alter cognitive-motor performance, including sleep deprivation [129], hypoglycemia [130], and other physiological stressors [131].

Thus, there is no clear threshold established at what magnitude of DEH (e.g., $> 2\%$ BML) cognitive-motor impairments begin to occur. Because 2% BML elicits physical (e.g., aerobic) performance decrements [17] along with accompanying physiological compensation due to hypovolemia, increased plasma osmolality [18], some suggest cognitive-motor impairments also begin to arise in parallel. Experimental evidence indicates impairments occur at 2% with exacerbated decrements in cognitive-motor functions at 4% BML [70, 71], suggesting an association between body water deficits and impaired mental functioning. However, subsequent studies have not always supported this relationship, with a recent review [30] suggesting additional protective mechanisms for the brain to preserve cognitive-motor functions until DEH reaches a higher threshold (e.g., $\geq$3% BML).

Therefore, my purpose was to perform a systematic review of the literature and utilize a quantitative technique (i.e., meta-analysis) to determine the impact of DEH on performance of cognitive-motor tasks. My primary aim was to examine potential experimental design factors (e.g., method to elicit and magnitude of DEH, and cognitive-motor test selected) that may influence the effect size estimate. I hypothesize that DEH will induce a significant impairment in cognitive-motor performance. Second, I hypothesize that the magnitude of DEH will be significantly associated with the degree of cognitive-motor impairment, observable at a minimum threshold ($> 2\%$ BML) similar to that of other physical performance measures.

2.3 Methods

A systematic review was conducted on the research literature for the effects of DEH and cognitive-motor performance. Cognitive performance was operationally defined as any measurable outcome resulting from completion of a cognitive-motor function task (e.g., reaction time, accuracy). The literature search was completed as of September 13, 2017. Searches were conducted in the following databases: PubMed, Medline, Psych Info, SportDiscus, ISI Web of Science, SCOPUS, ProQuest Theses and Dissertations, which collec-

tively returned 8306 results (6591 without duplicates). References from relevant review articles were also examined [86, 72, 132, 30] for articles not uncovered previously. Search terms consisted of: (*hydration OR water loss OR weight loss OR hypovol* OR sweat loss) AND (cognition OR cognitive-motor function OR cognitive-motor performance OR executive function OR response time OR reaction time OR intelligence OR memory OR mood OR vigilance OR pattern recognition OR letter* OR processing) AND (adult* OR college student).

Inclusion Criteria

Studies meeting the following inclusion criteria were considered for review: i) the study was conducted on healthy (i.e., no clinical conditions) adults ($\geq$ 18 y), ii) the study contained at least two time-points (within or between groups) when cognitive-motor testing was completed following DEH and under a control condition, iii) changes in hydration status were reported with body mass loss, and iv) cognitive-motor performance variables (e.g., accuracy, reaction time) were reported. Studies not on healthy adults and those inducing chronic dehydration (beyond 72 h) were excluded from the analysis.

Selection of Studies

A total of 6591 relevant publications were originally identified through the database searches. Of those, 6512 were initially excluded based on title and/or review of the abstract (PRISMA diagram, Figure 2.1). Therefore, the full text of 79 publications was reviewed for meeting the inclusion criteria. Of those 79, 48 were excluded due to no control condition, weight loss induced by $>$3 d fluid restriction, no change of body mass measures, and no behavioral measures of cognitive-motor performance resulting from DEH. Our screening criteria resulted in a total of 32 articles to be included.

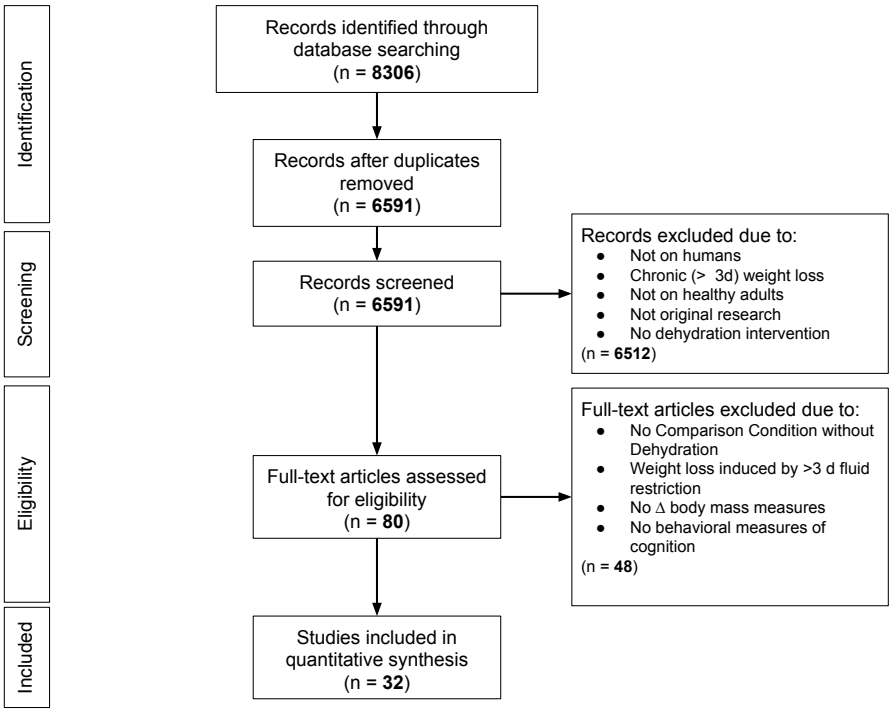

Figure 2.1: PRISMA diagram depicting the systematic review protocol in determining the population of studies for inclusion within the meta-analysis.

Data extraction

Studies included in the meta-analysis were independently coded by a minimum of two reviewers. Discrepancies in data entry were discussed and a consensus reached. Means, standard deviations, sample size, and correlations (if available) for both DEH and control conditions for all cognitive-motor tasks within the study were extracted. If a study included any treatment condition other than DEH and control, the data for those conditions were omitted. Each task was categorized into specific cognitive-motor domains of: executive function (attention, executive function specific tests, working memory), memory (short-term, long-term), information processing, motor coordination, or reaction time according to previously published criteria [124] and/or author description of the task. The executive function category was designed to capture all sub-components [133, 134] allowing for the examination of higher-order cognitive-motor processing. Cognitive outcome

variables (e.g., reaction time, accuracy) were extracted for all effect sizes. If the outcome variable of a given cognitive-motor test was not explicitly described as reaction time or accuracy, it was categorized according to the attribute most closely aligning (e.g., errors as accuracy, speed as reaction time). Study quality scores (e.g., PEDro) were not calculated as many of these studies omitted descriptions for specific design elements (e.g., blinding of subjects/therapists, group allocation) present in clinical trials for which these metrics were based upon.

Meta-Analysis

The extracted cognitive-motor performance data were converted to a standard format by calculating the standardized mean change score or effect size (ES) using the metafor package for R (*v1.9-9, www.metafor-project.org*). In studies where the correlational data were not reported, r was estimated from the median correlation taken from studies with i) known DEH-control correlations [79] and studies reporting effect sizes, means, and standard deviations from which r could be calculated [135, 5]. The known correlations (n = 15, range: 0.01 0.92) had a median r of 0.62. For the effect size estimate, Hedges g was employed to minimize the inherent bias of Cohens d to overestimate the effect size when standardized mean differences are used with small sample sizes [136]. For all analyses, a negative ES represents that DEH impaired cognitive-motor performance versus control conditions whereas a positive ES represents an improvement.

The studies in the meta-analysis assessed a wide array of cognitive-motor domains, providing several dependent outcomes (i.e., multiple tests with accuracy and reaction time) available to extract as results. Multiple effect sizes are problematic for most conventional meta-analyses, as the dependent structure of results (e.g., decreased reaction time but increased accuracy) may confound and compromise validity of the results unless the covariance structure is known [137]. Because of this, a multivariate (mixed-effects) meta-analysis was employed. Multivariate meta-analyses are appropriate when multiple related outcomes

are reported within each study (e.g., both reaction time and accuracy for a given test or multiple tests of executive function) and the dependence structure is unknown [138]. Multivariate meta-analysis, compared to other techniques, can control for multiple outcomes without necessitating study-wide averaging which can yield ES estimates that do not represent the range of study outcomes [138].

The meta-analysis was completed using the *rma.mv* function from the metafor package in R (*www.metafor-project.org*). The appropriate random effect structure was identified by fitting an intercept only model (no moderators) with multiple random effect configurations. Using the *anova* function within R, each different random effect configuration was compared. The best random effect structure was identified from the model yielding the lowest Akaike information criterion. This process resulted in a random effects model which allowed modeled between-study differences along with within-study differences based upon cognitive-motor domains assessed.

To assess the overall effects of DEH on cognitive-motor performance, an intercept-only model was used. Subgroup analysis was completed using the mods option within the *rma.mv* function. A Q test was instituted to examine if moderator variables significantly impacted the effect size estimates. If the subgroup was categorical (e.g., $\leq$ or $>$ 2% BML), the effect size estimates were compared to each other. If moderator variable was continuous (% BML), the slope was compared to zero using meta-regression. Publication bias of studies included within the meta-analyses was assessed using a Duval and Tweedie trim and fill correction funnel plot from the *trimfill* function within metafor. Because the trimfill function cannot analyze multivariate meta-analysis structures, a random effects meta-analysis model was utilized for this analysis. Across all comparisons, an alpha level of ≥ 0.05 was used to indicate statistical significance. As is common practice, ES (Hedges g) of 0.2, 0.5, and 0.8 were considered small, moderate, and large, respectively, while ES < 0.1 considered trivial [139].

2.3.1 Subgroup Analyses

We aimed to determine the influence of experimental factors on the overall ES using moderator analysis. A subgroup meta-analysis (i.e., meta-analyses comparing subsets of studies) was used to probe potential moderator variables such as the type of cognitive-motor domain, type of performance outcome, method of DEH, or magnitude of DEH. In order to be a subgroup, a minimum of five studies in the category was required. Therefore, motor coordination was not included as a cognitive-motor domain because of insufficient data. A majority of studies (m = 22) assessed multiple domains of cognitive-motor performance. The cognitive-motor domains compared were executive function, information processing, memory, and reaction time. Outcome variable types (accuracy and reaction time) were also compared against each other. A clear majority of tasks provided both accuracy and reaction time outcomes.

Methods to induce DEH were coded into the following categories: exercise, heat exposure (ambient temperature $\geq 27^{o}$C), exercise-heat stress (exercise + heat exposure with ambient temperature $\geq 27^{o}$C), or fluid restriction. Two studies [76, 80] induced DEH via both an exercise only and exercise plus diuretic trial. The presence of a diuretic did not significantly increase BM loss; therefore, both trials were averaged for the analysis. Both studies were subsequently categorized as exercise-heat stress protocols. Another two studies utilized fluid restriction plus exercise to induce DEH [105, 135]. One study utilized a 15 h fluid restriction protocol followed by 45 min cycling at $\sim$70% maximum effort in a temperate environment and was therefore classified as an exercise protocol [105]. The other study [135] had subjects undergo a prolonged fluid restriction protocol before one measured condition and then an exercise bout. In that case, the data point following exercise was classified as an exercise protocol. DEH methods were also categorized into two classifications: with/without the addition of environmental heat stress and with/without exercise. The magnitude of DEH was also sub-grouped into a cut point of $\leq$2 or $>$2 %BML to examine whether cognitive-motor studies inducing sufficient body water losses typically

observed to elicit physiological compensation [50] had greater impairments. If information was provided about subject fitness level (both aerobic exercise testing or author descriptive information), this information was used to categorize subjects as sedentary, recreational, or high fitness (VO2 max $>$ 55 mL/kg/min). In the presence of a significant Q value, pairwise comparisons were made between different levels of the moderator variable with Bonferroni-Holm corrections.

2.3.2 Meta Regression

The magnitude of DEH (values ranging from 1.1 6.0% BML across individual studies) associated with cognitive-motor task impairment was examined using meta-regression. Because each specific BML was coded, multiple levels of DEH per study were possible, even with small differences (e.g., 2.1 vs 2.2 %). If not reported, BM measures were converted to a percent change score (%BML = (BMpost - BMpre) / BMpost).

2.4 Results

2.4.1 Study Characteristics

Table A.1 presents the characteristics of each study in the analysis. The final sample consisted of 32 studies (m), all were published in peer reviewed journals except one [81] found in ProQuest. In total, there were 397 subjects and 258 effect sizes (k) with a median of 6 effect sizes per study (range: 1-36). All studies utilized a repeated measures (within subject) design. Across all studies, the median BML incurred was 2.0% (min-max: 1.1 - 6.2%). Because DEH magnitude was determined based on %BML, nine studies had multiple levels within the study compared to 23 studies eliciting only one level of DEH. Sixteen studies elicited DEH via exercise-heat stress, twelve using exercise only (no heat), five with heat stress only (no exercise), and four with fluid restriction only. Three studies utilized multiple methods of DEH. The cognitive-motor domains assessed following DEH were executive-functions (m = 24; mental math, trail-making test, proof reading, grammatical

reasoning, map recognition, logical relation test, visual vigilance, test of variables of attention, monotonous driving task, digit span, match to sample, n-back test), reaction time (m = 15; simple/choice reaction time), memory (m = 18; repeated acquisition, story recall, word recognition, map recall, picture recall), information processing (m = 8; perceptive discrimination, target evaluation, critical flicker fusion test, substitution test, visual perception test, letter-digit substitution), and motor coordination (m = 4; unstable tracking, psychomotor test, Groton maze chase). Of the 32 total studies, 27 (84%) included only male subjects. When fitness level was measured/described, only recreationally (m = 13) or highly fit (m = 13) subjects were included (6 studies unspecified). Approximately half of the studies (17 of 32) reported at least one significant cognitive-motor impairment following DEH.

2.4.2 Overall Effect of Dehydration on Cognitive Performance

Figure 2.2 presents ES for all studies reporting effects of DEH on cognitive-motor performance. Considerable variation was observed among studies, with individual study-averaged ES ranging from -1.25 to 0.75. Nine studies (28%) demonstrated a study-averaged positive ES or improvement in cognitive-motor performance (one was significant) while twenty-three studies (72%) had negative ES (eight were significant). When including all studies and outcomes (m = 32, k = 258), DEH elicited a small but significant impairment in cognitive-motor performance (g = -0.22, z = -4.1, $p < 0.0001$, 95% CI: [-0.33, -0.12]). There was significant heterogeneity across studies (Q(257) = 683.8, $p < 0.0001$). The amount of total variance attributed to the total amount of within-study heterogeneity was low to moderate (I^2 = 37.6%) while between study heterogeneity was low (τ^2 = 0.13). The trim and fill analysis (Figure 2.3) suggested three studies observing a strong positive effect (g of $\sim$ 0.5, 0.6, 0.9) of DEH on cognitive-motor performance were calculated to be included in order to minimize publication bias. However, the addition of three positive effect size studies did not alter significance of the overall ES (g = -0.15, 95% CI: [-0.29, -0.03], p = 0.02), although ES was reduced to between small and trivial. It is also highly

unlikely that such "theoretical" positive improvements in cognitive-motor function due to DEH would have remained unpublished. A subgroup meta-analysis comparing published studies versus unpublished studies was not possible since only one study was included within the unpublished group.

2.4.3 Analysis of Moderator Variables

Cognitive Domains

Table 2.1 presents moderator variables for studies examining the effects of DEH on cognitive-motor performance. DEH elicited a significantly greater impairment ($Q(1) = 7.9$, $p = 0.005$) in accuracy compared to reaction time outcomes across the range of cognitive-motor tests in all studies. There was a significant effect ($Q(3) = 13.9$, $p = 0.003$) of DEH on the cognitive-motor domains assessed, with significantly ($p < 0.001$) greater impairment in tasks emphasizing executive functions compared to reaction time tasks, although no other significant differences were found ($p > 0.05$) among the other categories (i.e., information processing, memory, and reaction time tasks). Motor coordination ($m = 4$, $k = 32$) tasks also showed a significant ($p = 0.0002$) impairment ($g = -0.47$, 95% CI: [-0.72, -0.22]). Figure 2.4 presents the relationship between effect size estimates for tasks of executive functions ($m = 24$, $k = 124$) and those based upon reaction time ($m = 15$, $k = 46$).

Level of Dehydration

Figure 2.5 presents the meta-regression results for all effect size estimates across the range of DEH. Overall, there was a significant association between magnitude of DEH (% BML) (slope = 0.07, $Q(1) = 4.1$, $p = 0.03$) with decrements in cognitive-motor performance. Across all cognitive-motor domains and outcomes, studies eliciting a BML $>2\%$ elicited significantly greater ($Q(1) = 5.2$, $p = 0.03$) cognitive-motor impairment than studies eliciting $\leq 2\%$ BML, although ES estimates for both sub-groups were significantly different from zero (Table 2.1). The level of DEH was not significantly associated with decrements

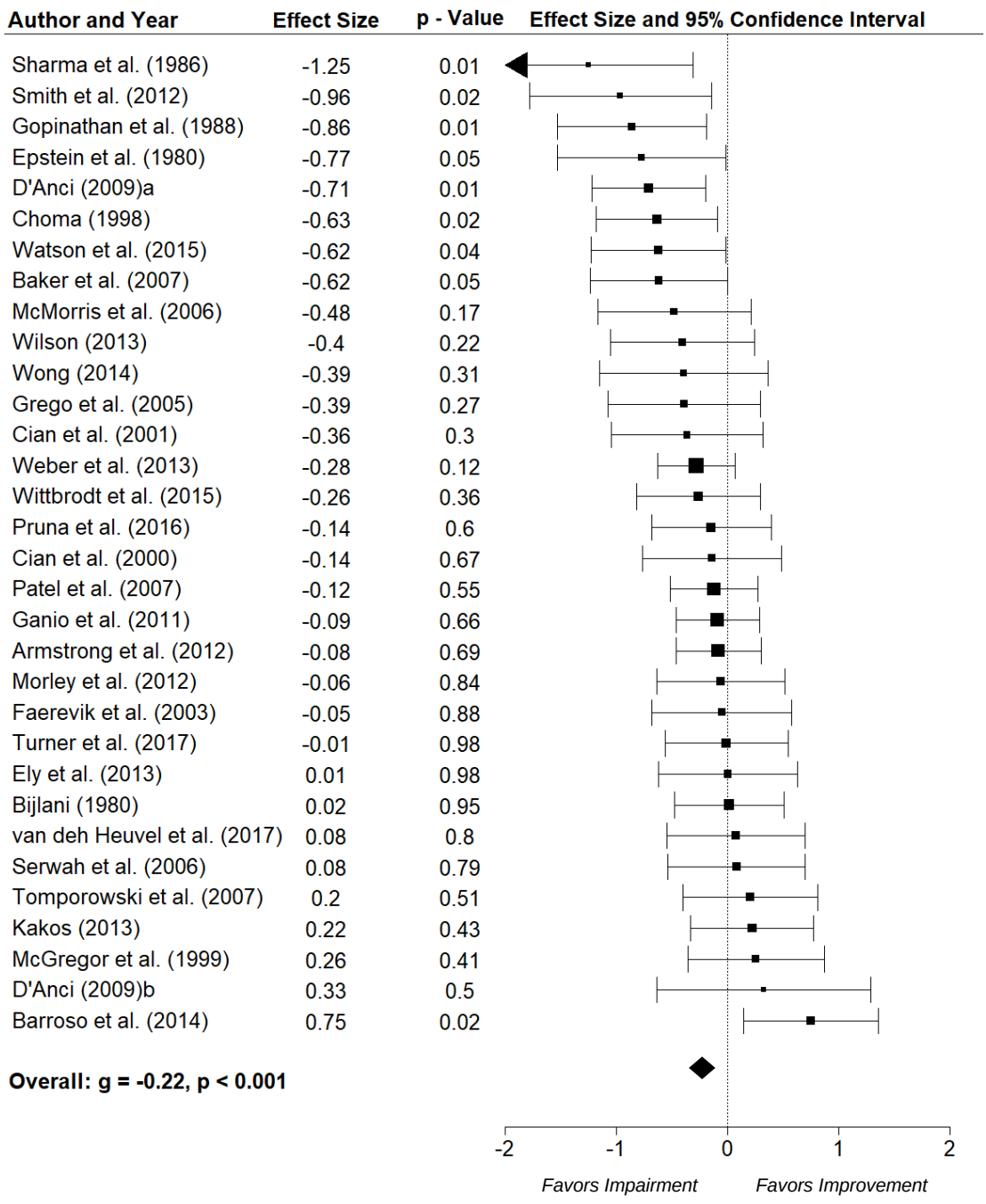

Figure 2.2: Forest plot of effect size (ES) for all studies (m = 32) examining dehydration on cognitive-motor performance. Negative ES (g) indicate dehydration impaired cognitive-motor performance whereas positive effect size (g) indicates improved cognitive-motor performance. Box size indicates the relative weight of each study attributed to overall ES and horizontal lines indicate 95% confidence intervals. Diamond indicates mean overall effect size with width corresponding to the 95% confidence interval.

Table 2.1: Effect of categorical moderators for the impact of dehydration on cognitive-motor performance. Q Test P value: difference within a category, g: effect size, 95% confidence interval (CI) of the effect size, p value for the individual moderator compared to zero (p), m: number of studies, k: number of outcomes. *significantly ($p < 0.05$) different effect size within cognitive-motor domain compared to attention effect size.

Variable	**Q Test P Value**	**g**	**95% CI**	**p**
Outcome Variable	**0.005**			
Accuracy (m = 30, k = 181)		-0.26	[-0.38, -0.15]	<**0.001**
Reaction Time (m = 24, k = 77)		-0.13	[-0.26, -0.01]	**0.04**
Dehydration Magnitude	**0.02**			
≤2% Body Mass Loss (m = 18, k = 135)		-0.13	[-0.27, 0.00]	**0.05**
>2% Body Mass Loss (m = 19, k = 123)		-0.30	[-0.43, -0.17]	<**0.001**
Cognitive Domains	**0.003**			
Executive Functions (m = 24, k = 124)		-0.28	[-0.39, -0.16]	<**0.001**
Information Processing (m = 8, k = 28)		-0.11	[-0.29, 0.07]	0.24
Memory (m = 12, k = 48)		-0.16	[-0.31, -0.01]	**0.03**
Reaction Time (m = 15, k = 46)		-0.09*	[-0.22, 0.04]	0.16
Method of Dehydration	0.47			
Exercise (m = 11, k = 43)		-0.22	[-0.39, -0.06]	**0.01**
Heat Stress, No Exercise (m = 5, k = 31)		-0.18	[-0.41, 0.06]	0.14
Exercise + Heat Stress (m = 16, k = 175)		-0.20	[-0.35, -0.06]	**0.005**
Fluid Restriction (m = 4, k = 9)		-0.44	[-0.73, -0.14]	**0.004**
Subject Fitness	0.41			
Recreationally Fit (m = 13, k = 128)		-0.30	[-0.46, -0.14]	**0.0002**
Highly Fit (m = 12, k = 84)		-0.18	[-0.35, 0.00]	0.06
Not Specified (m = 6, k = 44)		-0.12	[-0.38, 0.15]	0.38

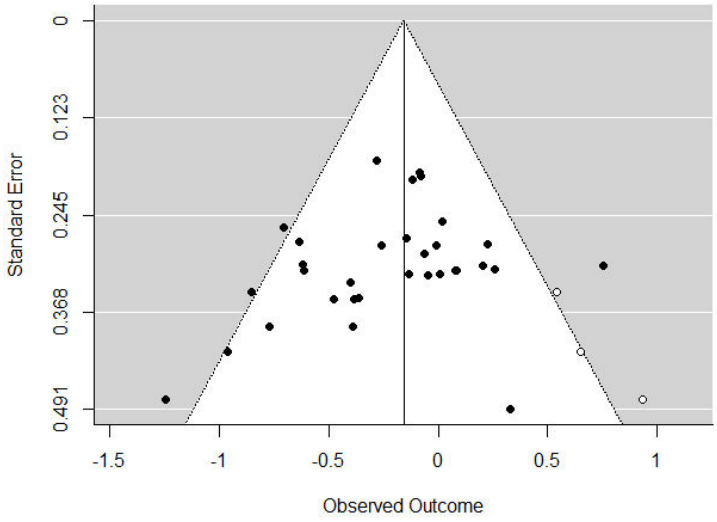

Figure 2.3: Trim and Fill plot for the meta-analysis. Open circles indicate studies required to minimize publication bias.

in the broader category of executive functions (k = 124; slope = 0.06, p = 0.23); however, $>$2%BML (m = 15, k = 70; g = -0.48, 95%CI: [-0.68, -0.29]) impaired executive functions to a greater extent than $\leq$ 2% (m = 17, k = 100; g = -0.23, 95%CI: [-0.39, -0.06]; Q(1) = 6.1, p = 0.01).

Other Factors

No significant differences in ES were observed between methods to induce DEH (Q(3) = 2.5, p = 0.47). Three methods (exercise, exercise-heat stress, and fluid restriction) elicited significant ($p < 0.05$) cognitive-motor impairment while heat stress alone did not (p = 0.13). Furthermore, cognitive-motor performance was not impaired to a greater extent (Q(1) = 0.38, p = 0.54) when DEH was elicited with an element of environmental heat stress (heat stress or exercise-heat stress) compared to protocols in neutral temperatures (exercise, fluid restriction). Likewise, using exercise to induce DEH (exercise, exercise-heat stress) did not (Q(1) = 0.08, p = 0.77) exacerbate cognitive-motor impairments compared to resting fluid restriction protocols (Table 2.1). Level of fitness (Q(2) = 1.8, p = 0.40) did not appear to influence results although recreationally fit (p = 0.0002) subjects experienced significant

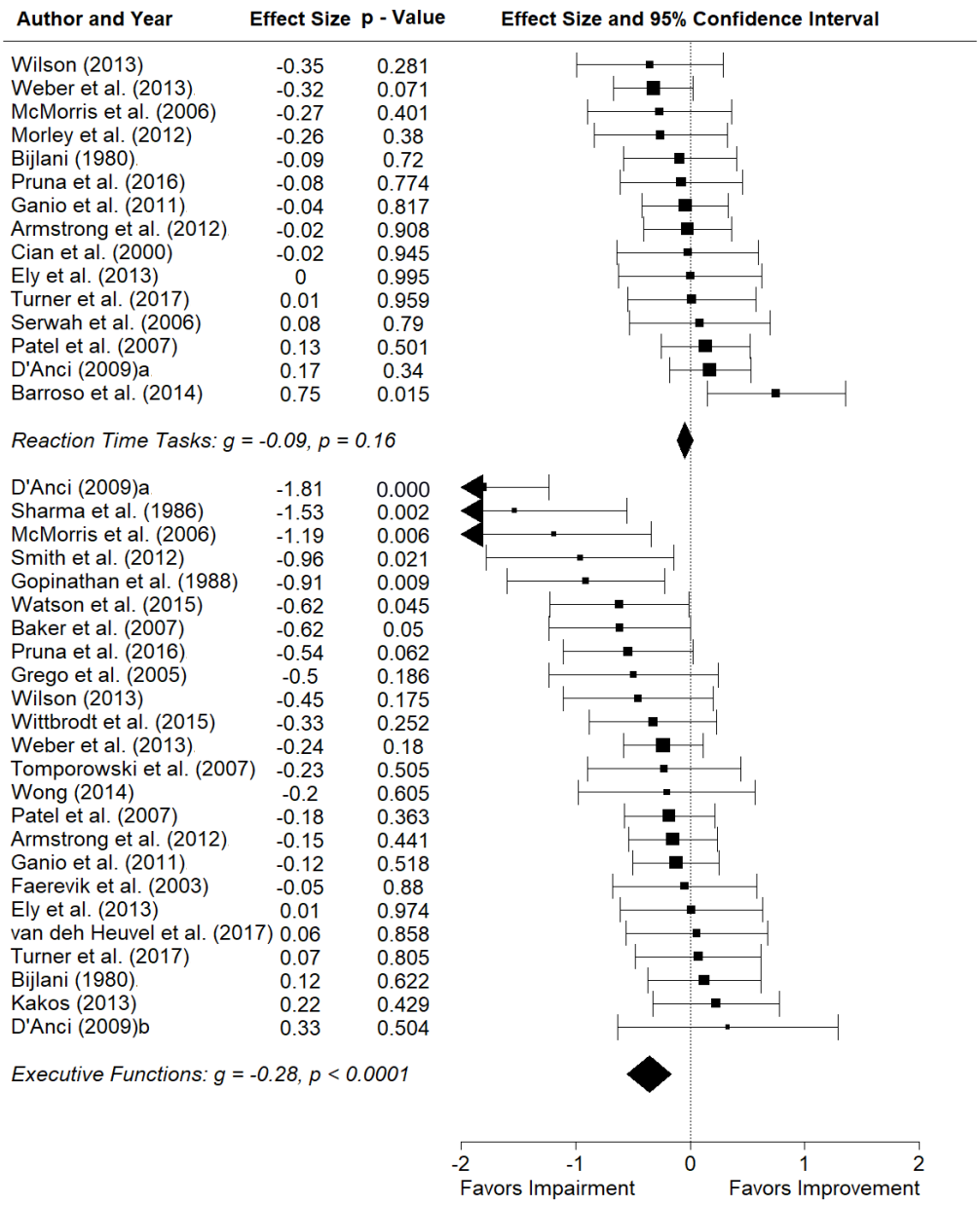

Figure 2.4: Forest plot of studies examining sub-group analysis for the effect of dehydration on tasks utilizing different cognitive-motor domains: executive functions (bottom) and lower order cognitive-motor processing (e.g., simple/choice reaction time, top). The two effect sizes were significantly different from each other ($Q(1) = 15.6$, $p < 0.001$). Negative ES (g) indicate dehydration impaired cognitive-motor performance whereas positive effect size (g) indicates improved cognitive-motor performance. Box size indicates the relative weight of each study attributed to overall ES and horizontal lines indicate 95% confidence intervals. Diamond indicates mean overall effect size with width corresponding to the 95% confidence interval.

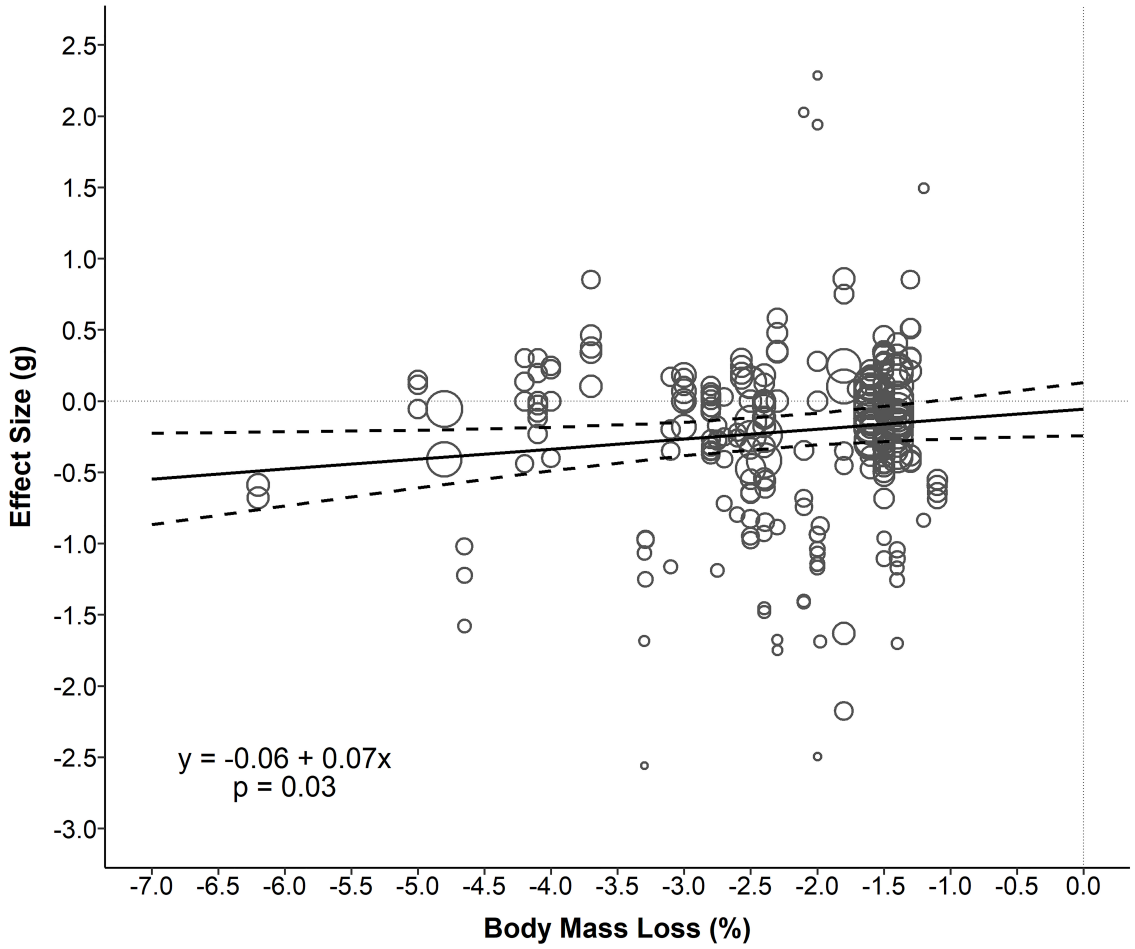

Figure 2.5: Meta-regression analysis for the magnitude of body mass loss (%) and effect size (g) for cognitive-motor task performance (negative values indicate impairment). Each outcome (n = 258) for all cognitive-motor test variables is depicted by a circle, with the circle size representing the relative weight attributed to each effect size. The slope for the line of best fit (solid line) was significantly different from zero (p = 0.04). Dashed lines indicated 95% confidence interval around line of best fit.

cognitive-motor impairment following DEH but highly fit subjects did not (p = 0.06; Table 2.1).

2.5 Discussion

Dehydration is believed to impair cognitive-motor performance and potentially increase workplace accidents and occupational risk [7]. Although narrative reviews suggest dehydration may impair cognitive-motor performance [73, 86], previous research has not unequivocally supported this position [18]. The current study employed a quantitative analysis of studies and objectively determined there is a significant effect of dehydration on cognitive-motor performance. We also assessed the influence of several study design factors that contribute to this this effect. Our meta-analysis supports previous hypotheses [77, 30] that higher-order cognitive-motor domains (i.e., executive function) are more likely to degrade with DEH compared to lower-level tasks (i.e., reaction time) and the degree of cognitive-motor impairment is associated with the magnitude of DEH.

The main finding of this study is that dehydration elicits a small but significant impairment on cognitive-motor performance. This negative effect size aligns with narrative reviews suggesting dehydration may mirror the effects of other nutritional interventions by altering cognitive-motor performance but only by a small degree [72]. Furthermore, this significant finding occurred in the face of significant study heterogeneity, which has been repeatedly acknowledged in narrative reviews [73, 72], thus making a firm conclusion challenging. To this point, a minority of studies (only 24%) observed statistically significant cognitive-motor impairments following dehydration. Our meta-analytic technique overcomes this limitation and enhances the ability to assess the impact of various experimental factors which potentially contribute to this heterogeneity.

The second main finding of the current study was that dehydration does not affect all cognitive-motor domains equally. Previous studies have demonstrated this experimentally [75, 105, 76] by observing significant cognitive-motor impairments following DEH

in some, but not all, cognitive-motor domains. Some have also suggested higher-order cognitive-motor domains may be impaired to a greater extent following dehydration [77, 73, 86, 30], although a proposed mechanism has not been identified. This is the first comprehensive analysis to systematically demonstrate which cognitive-motor domains may be more at risk following dehydration. We observed that tasks requiring executive functions were significantly more impaired following dehydration compared to those investigating reaction time. Disentangling executive function impairments is difficult, as deficits may occur in multiple cognitive-motor domains required for task completion (e.g., inhibition, attention) [140]. Previously, dehydration (-1.6% BML) elevated fronto-parietal brain activations during an executive function task (Tower of London) but without significant performance impairments [46]; thus, hypothesizing that dehydration elicits neural inefficiencies. Furthermore, because fronto-parietal activations appear integral to executive functioning [140], prolonged cognitive-motor processing (required for tests of attention), may also be responsible for executive function impairments. The specific rationale as to why other cognitive-motor domains are less affected is yet to be understood. Specific cognitive-motor domains may require different brain regions and neurotransmitter systems for adequate processing [86], potentially making some brain areas (and cognitive-motor domains) more susceptible to body water deficits.

Along with differences in the effect of DEH on cognitive-motor domains, I found accuracy is impaired to a greater extent than reaction time outcomes. This may suggest a change in strategy to preserve performance. One study demonstrated this experimentally following prolonged cycling, observing increased errors with dehydration compared with faster reaction time [141] often referred to as the speed-accuracy trade-off [142]. An alternate explanation is that dehydration simply impairs higher-order cognitive-motor processes involved in decision making but, for reasons currently unclear, still elicit responses with similar temporal characteristics. Future studies might investigate how dehydration alters the speed-accuracy cognitive-motor strategies.

Another major finding of this study was that the decline in cognitive-motor performance was directly related to the magnitude of water deficit (e.g., body mass lost). This finding is in agreement with individual studies observing this graded phenomenon [70, 71], but differs from others that have elicited large body mass losses ($\sim$4% BML) without cognitive-motor performance impairments [13, 127]. Furthermore, greater cognitive-motor impairments were observed in studies eliciting a dehydration threshold sufficient to induce physiological compensation ($>$2%) versus when compensation was unlikely ($\leq$ 2% BML) [18]. Taken together, these findings suggest the hypovolemia and/or hypertonicity (and subsequent physiological compensation) elicited by dehydration may at least be partially responsible for cognitive-motor impairments and this effect is observed at increasingly greater body mass losses. However, the mechanisms responsible for this effect are not entirely clear. It is well accepted that human cognitive-motor capacity is limited [143]. If task demand exceeds this capacity, performance will decline. It is possible, then, that progressive body water deficits may incrementally limit cognitive-motor capacity resulting from altered neurotransmitter levels [86], or brain structures [46] known to be associated with degraded cognitive-motor performance in aging and/or disease states [144].

Our analysis also ruled out several other factors which may influence the impact of dehydration on cognitive-motor performance. Because increased fitness may increase cerebral circulation and brain perfusion [145], it was believed highly fit subjects may be more resilient to cognitive-motor decrements following dehydration. However, sedentary individuals were not recruited in these studies, or compared specifically to recreationally or highly fit subjects. Future studies might investigate this factor with sedentary versus highly fit individuals using a non-exercise dehydration protocol. The method utilized to achieve dehydration was also not different, although passive heating (without exercise) did not elicit significant cognitive-motor impairments. This finding conflicts with narrative reports suggesting heat stress may be required to elicit cognitive-motor deficits [30]. Some have suggested that, when dehydration is coupled with exercise and/or heat stress, the true effect

of dehydration is confounded [86]. Effect size estimates for all dehydration methods were small to moderate ($\sim$ -0.2 to -0.5), suggesting any obfuscation of the true effect of dehydration on cognitive-motor performance by multiple physiological stressors is likely minor. Furthermore, three of the 32 studies [75, 74, 135] have investigated multiple dehydration methods within a single study testing the same subjects and concluded similar results. Two studies reported cognitive-motor impairments with both exercise and heat stress alone [75, 74] and one found no difference when either fluid restriction or fluid restriction was combined with exercise [135]. While not directly assessed in this study, dehydration-mediated cognitive-motor impairments may be influenced by affective changes such as altered mood [76, 80], increased feeling of mental exertion [85], or the presence/sensation of thirst [101, 146]. Mental exertion may also be elevated during cognitive-motor testing [107, 5], however, this has not always paralleled performance impairments.

As has been suggested previously, meta-analyses have some inherent limitations [136]. First, not all cognitive-motor domains (motor coordination) were included in the subgroup analysis given too few studies ($n < 5$) and thus merits future investigation. Secondly, some studies [147, 14, 102] omitted reporting data from non-significant tests following dehydration. It is also possible that not all studies were of similar quality in terms of randomization, double blinding, and convenience sampling. Thus, the impact of these factors on the current meta-analysis is unclear, and I acknowledge results may change as future studies appear in the literature.

2.6 Conclusion

In conclusion, I have identified that, despite a myriad of studies using different experimental protocols reporting a variable range of results, dehydration elicits a small, but significant impairment in cognitive-motor performance. Furthermore, executive functions that require an accurate response appear more susceptible to impairment following dehydration compared to other cognitive-motor domains involving lower order mental processing (e.g.

simple reaction times). The magnitude of dehydration is associated with the impairment in cognitive-motor performance, specifically notable when $> 2\%$ body mass loss. Thus, the threshold for the impact of dehydration on cognitive-motor performance may be similar to that previously reported for the performance of physical exertional tasks (i.e., exercise).

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CHAPTER 3

AIM 2: DEHYDRATION AND EXERCISE-HEAT STRESS ALTERS BRAIN STRUCTURE, FUNCTION, AND IMPAIRS VISUOMOTOR PERFORMANCE

3.1 Abstract

Whether dehydration impairs performance of simple cognitive-motor tasks remains equivocal. This study determined the impact of prior exercise-heat stress with and without dehydration on brain structure, function, and performance during a rhythmic finger tapping task. Thirteen adults were assessed after cool-down from exercise-heat stress (EHS) either with or without water replacement ($\sim$3% body mass loss; EHS-DEH) compared to rest (CON). Anatomical scans and performance during a 20-min Visuomotor Pacing Task (VMPT) were evaluated concurrent with fMRI Blood Oxygen Level Dependent responses (n = 10). VMPT reaction time was not different across trials. EHS reduced VMPT accuracy vs CON (-7.2 $\pm$ 6.6%, p = 0.03); EHS-DEH reduced accuracy vs EHS (-8.2 $\pm$ 6.4%; p = 0.02) and CON (-15.3 $\pm$ 10.0%; p = 0.008). EHS elicited ventricular contraction (-5.3 $\pm$ 1.7%, $p < 0.0001$) and cerebellum enlargement (1.5 $\pm$ 0.8%; p = 0.0001) while EHS-DEH induced ventricular expansion (6.8 $\pm$ 3.4, $p < 0.0001$) and thalamus (-2.7 $\pm$ 1.3%, p = 0.005) and cerebellum (-0.7 $\pm$ 0.7%, p = 0.05) shrinkage vs. CON. Plasma osmolality was related ($p < 0.05$) to changes in thalamus (r = -0.45) and cerebellum volume (r = -0.61) which, in turn, were related ($p < 0.05$) to changes in lateral (r = -0.41) and fourth ventricle volumes (r = -0.67), respectively. EHS-DEH increased neural activation ($p < 0.05$) during VMPT within the thalamus, basal ganglia, temporal lobe, and insula vs EHS and CON. In conclusion, brain structural changes occur with plasma osmolality perturbations. Visuomotor performance is impaired by prior exercise-heat stress despite adequate water replacement, and further exacerbated with dehydration despite increased neural activation

during task completion.

3.2 Introduction

Dehydration is a common stressor and believed to adversely impact cognitive-motor and central nervous system function, despite inconclusive scientific evidence [16, 18]. Conflicting findings exist regarding the impact of dehydration on cognitive-motor performance, with some studies observing marked impairments [70, 71, 74, 148, 5], but many studies finding no differences [85, 14, 80, 13, 80, 79, 127], and one study finding improved performance [149]. No singular explanation accounts for these disparate results; but, explanations likely include inconsistencies within the assessed cognitive-motor domains coupled with study design considerations such as method to induce dehydration, magnitude of body water deficits, and combining dehydration with prior exercise-heat stress.

Previous studies examining the effects of dehydration on cognitive-motor function typically assess performance with computerized tasks utilizing a higher-order cognitive domain (e.g., executive control, information processing, memory) and requiring a response (e.g., button press). While these assessments hold value, they may assess overly broad cognitive-motor functions. Tasks isolating visuomotor responses allow researchers to examine the influence of a cognitive system (vision) on motor function through networks involving visual cortex, sensorimotor areas, parietal cortex, and basal ganglia [142, 150]. Some studies have suggested motor coordination may be impaired following dehydration [75, 74]. However, a primary component of visuomotor functioning, the ability to accurately process temporal information [151], has not been previously examined. Adequate visuomotor performance is essential to human-system interactions, and dysfunction (potentially from deficient visuomotor timing) might explain errant performance in tasks such as driving proficiency [5], pilot simulations [6], and sporting skills [103] following dehydration.

Whether acute neuroanatomical changes explain cognitive-motor impairments associated with dehydration remains unknown. Early animal autopsy studies observed no changes

in brain volume following severe dehydration (-10% of body mass, BM) [41] suggesting homeostatic neural mechanisms adequately offset large perturbations to body water balance [65, 61]. In contrast, subsequent human in vivo neuroimaging studies usually confirm total brain volume is not altered with dehydration [42, 45, 44, 48, 47]; however, lateral ventricle volume is observed to either expand [45, 46], shrink [44], or not change [42, 49, 47]. Lateral ventricle expansion is also associated with cognitive-motor decrements during aging and/or neurological disturbances and likely attributed to adjacent periventricular grey matter atrophy [152, 153, 154]. To date, only one study examined white and grey matter following dehydration, finding no volumetric change with 2% BM loss [49]. Furthermore, the other previous magnetic resonance imaging (MRI) studies have induced only nominal dehydration (¡ 2% BM loss) [46, 47], examined few subjects [42, 49], or have not controlled for prior exercise-heat stress [45, 44] when using this model to achieve dehydration.

In addition to anatomical analyses, MRI can assess brain function during cognitive-motor tasks. Nominal dehydration ($<$ 2% BM loss) purportedly elevates Blood Oxygen Level Dependent (BOLD) responses in fronto-parietal areas during completion of an executive function task, despite no impairment in task performance [46]. This suggests greater resources may be required to perform higher order cognitive-motor processing (i.e., neural inefficiency) with dehydration, although the impact on lower level tasks is unclear. Moreover, the impact of the exercise-heat stress (used to elicit dehydration) per se was not used as an appropriate control trial.

Thus, a comprehensive study is needed to examine whether body water deficits (3% BM) known to occur during occupational or military settings [1] change brain structures and alter brain function during a visuomotor task compared to when dehydration is prevented with water replacement. I hypothesized that, similar to a previous study [46], dehydration would elevate neural activity during a simple visuomotor task, however, performance would not be impaired. Secondly, I hypothesized that dehydration would alter brain structures, specifically by expanding ventricular volume as observed in some, but not all,

previous studies due to fluid movements across compartments. Furthermore, I hypothesized water replacement during exercise-heat stress would attenuate this structural change and preserve visuomotor performance without requiring greater neural activation.

3.3 Methods

3.3.1 Participants

All procedures and protocols were approved by the Georgia State University-Georgia Institute of Technology Joint Advanced Brain Imaging Institutional Review Board and conformed to the guidelines set forth in the Declaration of Helsinki. Informed and written consent were obtained before participation. Thirteen right-handed (six female) healthy adults (age: 23.6 $\pm$ 4.0 y, body mass: 61.3 $\pm$ 6.0 kg, body fat: 15.2 $\pm$ 3.0 %) participated in all three experimental trials of the study. All subjects engaged in regular exercise ($\geq$ 4 d/wk) and served as their own control by completing each trial. Due to available funding and technological complications, behavioral data was obtained on 13 subjects and imaging studies on ten.

3.3.2 Experimental Design

Subjects completed three preliminary sessions and three experimental trials, all within $\sim$3 weeks. Subjects were tested in Atlanta, GA, during non-winter months (late March through early December). Before all sessions, subjects were instructed to consume liberal ($>$ 500 mL) fluid the night before, abstain from alcohol and caffeine for the previous 12 h, and enter the laboratory after an overnight fast. Three preliminary sessions were conducted to establish baseline body mass (BM), plasma osmolality (POsm), and urine specific gravity (USG; ATAGO USA, Bellevue, WA) as previously recommended [18]. During one preliminary session, an exercise-heat bout was completed by subjects to estimate sweat rate and verify workload (treadmill velocity and grade) for the experimental trials.

Following the preliminary sessions, subjects completed three experimental trials: control (CON; no exercise-heat stress), exercise-heat stress with fluid replacement (EHS), and exercise-heat stress with dehydration (EHS-DEH; exercise-heat stress without fluid replacement). The order of experimental trials (EHS and EHS-DEH) was counterbalanced, but CON usually occurred first (n = 7) due to scheduling constraints in the MRI facility. Thus, I evaluated if there was any practice effect on behavioral variables based on test order.

The experimental trials were initiated in the morning ($\sim$0700) and first morning BM and USG were assessed to ensure adequate hydration status ($\leq$ 1% difference in BM from preceding 3 d average) [50]. Subjects then consumed a nutrition bar (250 kcal) and water (150 ml) 20 min before entering the hot (EHS, EHS-DEH; 45^{o}C, 15% RH) or temperate (CON; 22^{o}C, 30% RH) environments. For EHS and EHS-DEH, the exercise mirrored previously employed exercise-heat stress protocols [50, 13] consisting of 150 min of 45/15 min walk/rest cycles on a treadmill at $\sim$3.5 mph, 5% grade. The goal during EHS-DEH was to achieve $\sim$3% BM loss while minimizing muscular fatigue and high core temperatures. During EHS, subjects consumed a volume of water equivalent to sweat loss, while no water was consumed during EHS-DEH (only mouth rinse permitted once per hour). Following exercise, subjects moved to the temperate environment to cool for $\sim$30 min prior to a final BM, blood glucose, and POsm. Subjects then showered, changed into dry clothes, and transported to the MRI facility with a total recovery period (end of exercise to beginning of scans) of 45 min. During CON, subjects reported under the same baseline conditions (water, meal) and sat quietly in the temperate environment while abstaining from mentally stimulating activities before being transported for scanning at the same time of day ($\sim$1100).

3.3.3 Physiological and Perceptual Measures

Blood samples were obtained by finger puncture before and after exercise on a heated digit after being seated for 10 min using freeze point depression (Osmette II, Precision Systems,

Natick, MA) as described previously [78]. Blood glucose was measured (OneTouch UltraMini, LifeScan Inc., Wayne, PA) post-exercise for EHS-DEH and EHS and 90 min after the meal for CON. Nude, dry BM was measured before and after each hour period of exercise on a digital platform scale. Rating of thirst (1-10 Likert scale) were also assessed at five min intervals.

3.3.4 MRI Scanning and Visuomotor Task

Subjects were placed in the 3T MRI (Siemens Trio, Siemens, Germany) scanner with the 12-channel head coil affixed and head position in a way to minimize movement in the X, Y, or Z axes. The scanning sequence consisted of a T1-MPRAGE with 256 slices and 1.0 x 1.0 x 1.0mm voxel size (TA: 6.17s, 9^o flip angle, TI: 850ms, TR: 2250ms; TE: 3.98ms) and a T2 Space with 1.0 x 1.0 x 1.0mm voxel size (256 slices, TA: 4.43s, TR: 3200ms, TE: 428ms). In between the T1 and T2 scan, subjects completed the visuomotor pacing task (described below) during which blood oxygen level dependent (BOLD) responses were measured using an echo-planar imaging sequence with a total of 714 volumes (37 slices; TR = 2000 ms, TE = 30 ms, flip angle = 90^o, field of view = 204 x 204 mm^2, in-plane resolution of 3 x 3 mm^2, slice thickness: 3.0 mm). Each fMRI scanning block lasted approximately 11 minutes.

During the functional MRI (fMRI) scanning, subjects completed a visuomotor pacing task (VMPT; E*Prime, Psychology Software Tools, Sharpsburg, PA) requiring visually-paced rhythmic finger tapping with the right index finger. In the scanner, subjects lay supine and viewed a display monitor (Silent Vision 6011, Avotec, Stuart, FL) via a mirror placed on the head coil. Headphones (Silent Scan 3100, Avotec, Stuart, FL) were placed on the subject and adequate visibility of the monitor was confirmed before each scan. If required, vision was corrected using MRI-compatible lenses. Due to budget restrictions, several subjects (n = 3) did not undergo MRI scanning, and instead completed the VMPT in a MRI simulator (Psychology Software Tools, Sharpsberg, PA) built to mimic conditions

within the MRI scanner (i.e., supine position, enclosed space, head coil).

The VMPT consisted of 1 Hz alternating stimuli (yellow square presented for 500 ms) and fixation crosses (i.e., interstimulus interval) with two pacing variations: i) regularly paced (VMPTr; fixation cross for 500 ms) and ii) irregularly paced (VMPTi; fixation cross presented for 400-600ms). Subjects were instructed to respond to the stimulus (yellow square) by pressing a button box (FORP 4 Diamond, Current Designs, Philadelphia, PA). Errors were encoded binomially: '0' (missed response) or '1' (correct response). Blocks of thirty stimuli (all either VMPTr or VMPTi) were followed by 30 s rest. Twenty total blocks were completed (n = 600 stimuli) with extended (120 s) rest periods every five blocks, with total test duration equaling $\sim$22 min. Block presentation (VMPTr or VMPTi) was randomized for each test iteration. Two behavioral measures were examined: accuracy (percentage correct responses) and reaction time (stimulus presentation to button press). The nature of the VMPT dictated that only correct responses could be examined for reaction time. Both reaction time and accuracy were averaged across 5-min blocks of time. Previous research has identified tasks with parameters of the VMPT (i.e., 1 Hz single digit finger tapping with one stimulus-response combination) are not affected by learning or trial order [93].

3.3.5 Anatomical Analyses

Cortical reconstruction and volumetric segmentation were performed using the Freesurfer image analysis pipeline (surfer.nmr.mgh.harvard.edu). Briefly, the pipeline involves cortical surface extraction and "skull-stripping" (removal of extracerebral voxels), grey/white matter segmentation based on intensity differences and geometric structures, computing planes to anatomically disconnect the two hemispheres and subcortical structures, computing a pial surface (smooth grey-white matter interface), and correcting inter-individual topological defects in surface by computing a spherical topology [155, 156]. Furthermore, because dura and grey matter are difficult to distinguish with a T1 image, a T2 scan was

provided to refine the estimate of the pial surface and registered with the T1 image using boundary-based registration [157] provided as part of FreeSurfer (surfer.nmr.mhg.harvard/fswiki/FsFast). The Freesurfer anatomical pipeline is capable of detecting submillimeter differences between groups and has an inter-class correlation of > 0.95 and reproducibility > 0.99 in measuring lateral ventricle volume [107]. Because of large inter-individual variability in some brain regions (e.g., lateral ventricles) [158], a relative change (compared to CON) was computed for each area (% change = (([trial] CON) / CON) * 100).

3.3.6 BOLD Analysis

fMRI data analysis was completed using FSL (www.fmrib.ox.ac.uk/fsl). All data were preprocessed by motion correcting images (MCFLIRT) [159], removing non-brain tissue (BET) [160], distortion-corrected with a fMRI field map using PRELUDE and FUGE [161], spatially smoothed using a Gaussian kernel of 8 mm full-width half maximum, and high pass temporal filtering (sigma = 100 s). First level fixed effects (time-series) analysis was completed with a generalized linear model (FILM) including nonparametric estimation of time series autocorrelation [162]. fMRI data for each subject were analyzed in native space (i.e., individual subject brain) before being initially registered to their own high resolution structural image and then subject to a nonlinear registration to standard MNI space (MNI152, Montreal Neurological Institute; Montreal, Quebec, Canada) using FNIRT. All blood oxygen level dependent (BOLD) signals were measured as signal intensity compared to the rest periods. Time series analysis was completed with the contrasts of the entire task (VMPTr and VMPTi) along with further analysis isolating VMPTr and VMPTi.

Second level analyses (i.e., across subjects and sessions) were completed using mixed effects (FLAME 1+2) which uses Markov Chain Monte Carlo sampling to identify true random-effect variance and degrees of freedom for each voxel. The main analysis examined the BOLD responses of the VMPT, VMPTr, and VMPTi during each session, and was restricted to grey matter voxels. To compare across trials, Z-statistic images were produced

by applying a cluster threshold of $Z \geq 2.5$ and (corrected) cluster significance threshold of $p = 0.01$ [163]. Contiguous clusters were identified using the Z statistic and then compared with the cluster probability threshold. Significant clusters were binarized and fed into the atlasquery function of FSL. Cluster peaks and anatomical locations were localized in MNI152 space using the Lateralized Harvard-Oxford Cortical Structural Atlas within the cortex and Harvard-Oxford Subcortical Structural Atlas for subcortical structures. Statistical maps were overlaid onto a standard brain template using MRIcron [164] with a threshold of $Z \geq 2.5$.

3.3.7 Statistical Analysis

Physiological and perceptual variables (POsm, BM, USG, thirst) were analyzed using a mixed model with repeated measures of trial (CON, EHS, EHS-DEH) and time (e.g., pre, post) within the nlme package of R (cran.r-project.org/web/packages/nlme). Relative change in brain areas were analyzed using a mixed model with repeated measure of trial. VMPT accuracy and reaction time were analyzed using a mixed model with repeated measures of trial, task version (VMPTr, VMPTi), and time block (first and last five minutes). When significant main or interaction effects were observed, post-hoc contrasts using Bonferroni-Holm corrections were calculated using the lsmeans package in R (cran.r-project.org/web/packages/lsmeans). Associations between changes in brain structures and plasma osmolality/VMPT accuracy were computed using one-tail (negative association) Pearson product-moment correlation coefficients. The alpha level was set a priori as $p \leq 0.05$ to indicate statistical significance. Data are presented as mean $\geq$ 95% Confidence Interval.

3.4 Results

3.4.1 Physiological Changes

Baseline hydration status was similar across all trials with no differences in USG (CON: 1.017 $\pm$ 0.003, EHS: 1.020 $\pm$ 0.003, EHS-DEH: 1.016 $\pm$ 0.003; $p > 0.05$) or POsm (CON: 283.8 $\pm$ 2.7, EHS: 283.6 $\pm$ 2.6, EHS-DEH: 284.5 $\pm$ 2.2 mOsm/kg; $p > 0.05$). Baseline BM was also consistent across trials ($p > 0.05$). As designed, EHS-DEH elicited significantly greater ($p < 0.001$) BM loss (-2.8 $\pm$ 0.3%) compared to EHS (-0.2 $\pm$ 0.3%) and was similar ($p = 0.46$) for men (-2.9 $\pm$ 0.4%) and women (-2.7 $\pm$ 0.4%). POsm increased (by 9.3 $\pm$ 2.1 mOsm/kg; $p < 0.001$) from baseline during EHS-DEH (293.8 $\pm$ 2.2 mOsm/kg) which was higher ($p < 0.0001$) compared to EHS (280.6 $\pm$ 2.6 mOsm/kg) and CON. POsm after EHS was also lower compared to CON (-3.2 $\pm$ 2.2 mOsm/kg, $p = 0.02$) and compared to baseline ($p = 0.03$). Post-exercise rating of thirst sensation was higher in EHS-DEH (5.8 $\pm$ 0.5) compared to EHS (1.4 $\pm$ 0.5, $p < 0.0001$). Post-exercise blood glucose was in a normal range (> 4.0 mmol/L) in all subjects, with no differences between exercise trials (EHS: 5.3 $\pm$ 0.8, EHS-DEH: 6.2 $\pm$ 0.7 mmol/L), although EHS was lower ($p = 0.001$) than CON (7.0 $\pm$ 0.8 mmol/L).

3.4.2 Visuomotor Performance

Reaction Time

When comparing the overall components of the VMPT test (collapsed across trial) , reaction time was slower for irregularly paced (VMPTi) versus regularly paced (VMPTr) intervals (by 16.6 $\pm$ 7.2 ms, $p < 0.0001$) and slower over time during the first 5 min block compared to the last 5 min block (by 22.3 $\pm$ 7.3 ms, $p < 0.0001$). Testing order across all three trials did not affect reaction time ($p = 0.38$). However, reaction time was not different among trials (CON: 168.9 $\pm$ 26.4, EHS: 159.9 $\pm$ 26.9, EHS-DEH: 160.2 $\pm$ 24.8 ms; $p = 0.54$).

Accuracy

To verify no learning effect, test order across all three sessions (irrespective of trial) did not impact (p = 0.65) VMPT accuracy (1: 83.5 $\pm$ 9.2, 2: 79.7 $\pm$ 10.9, 3: 78.6 $\pm$ 10.5 %). Accuracy was not different based on the test pacing intervals (VMPTr: 78.5 $\pm$ 13.8%, VMPTi: 75.7 $\pm$ 11.1%, p = 0.09) or a pacing interval by trial interaction (p = 0.28). Therefore, VMPT data were pooled across regular and irregular paced tasks. Over 20 min, accuracy was lower during EHS-DEH (69.7 $\pm$ 13.5%) compared to CON (85.1 $\pm$ 7.0%, p = 0.008) and EHS (77.9 $\pm$ 10.3%; p = 0.02). Accuracy was also lower during EHS compared to CON (p = 0.03). Figure 3.1 presents VMPT accuracy during the first and last 5-min time blocks across trials. As early as 5 min, EHS-DEH impaired accuracy compared to both CON (by -15.4 $\pm$ 9.3%, p = 0.003) and EHS (by -6.9 $\pm$ 6.2%, p = 0.03), and EHS compared to CON (by -8.5 $\pm$ 6.4%, p = 0.02). Accuracy was reduced over time from the first 5 min block compared to the last 5 min (by 9.2 $\pm$ 3.3%, p $<$ 0.0001), although no time by trial interaction was observed (p = 0.58).

3.4.3 Brain Anatomical Changes

Total brain volume (all tissues including cerebellum and ventricles but excluding the dura mater) was obtained from a data set of n =10 and not different among trials (p = 0.26). Total intracranial volume (total brain volume and sinus areas) was not different between CON and EHS-DEH (p = 0.96); but increased during EHS vs. CON (by 1.49 $\pm$ 0.8%, p $<$ 0.0001). The following brain structures were not significantly (p $>$ 0.05) altered by the experimental trials: total grey matter, cortical gray matter, cerebellar white matter, nucleus accumbens, amygdala, caudate, hippocampus, putamen, ventral dorsal column, brain stem, and choroid plexus.

Table 3.1 provides the brain structure changes associated with the experimental trials. EHS (vs. CON) increased (p $<$ 0.05) volumes of the cortical white matter, cerebellum, globus pallidus, and cerebellar gray matter (between $\sim$1 and $\sim$5%); but decreased ventric-

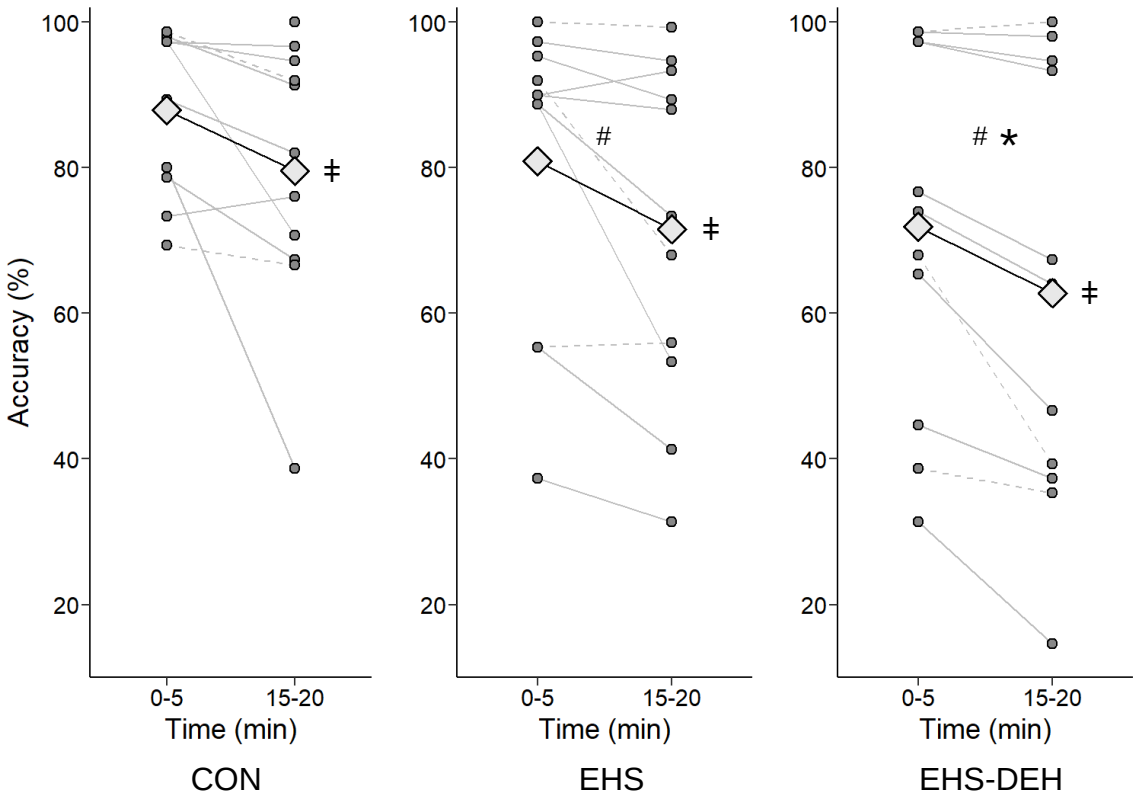

Figure 3.1: Mean accuracy (%) and individual responses (circles) for first and last five min time blocks of the visuomotor pacing task during resting control (CON), exercise heat stress with water replacement (EHS), and exercise heat stress coupled with dehydration (EHS-DEH; n = 13). Triangle shapes indicate subjects scanned in the mock MRI scanner. Symbols above mean (diamond) indicate trial effect (# $p < 0.05$ vs CON, * $p < 0.05$ vs EHS) and symbols in the right margin indicate a time effect (‡ $p < 0.05$ 15-20 min lower than 0-5 min)

Table 3.1: Mean 95% CI brain anatomical changes associated with the experimental trials (n = 10). Areas are expressed as the relative change from resting control (CON) for exercise heat stress without dehydration (EHS), and exercise heat stress with dehydration (EHS-DEH). * $p \leq 0.05$

	EHS vs CON (% Change)	**EHS-DEH vs. CON (% Change)**	**EHS-DEH vs. EHS (% Change)**
Aggregate Brain Areas			
Cortical White Matter	1.2 ± 1.1*	0.1 ± 0.9	1.1 ± 0.9*
Subcortical Grey Matter	0.5 ± 0.9	-1.1 ± 0.9*	1.5 ± 0.9*
Brain Structures			
Cerebellum	1.5 ± 0.8*	-0.7 ± 0.8*	2.2 ± 0.8*
Cerebellar Grey Matter	1.9 ± 0.9*	-0.7 ± 0.9	2.5 ± 1.0*
Corpus Callosum	0.5 ± 1.0	-0.8 ± 0.9	1.3 ± 0.9*
Subcortical Grey Matter			
Globus Pallidus	5.2 ± 5.1*	1.4 ± 3.3	-3.8 ± 3.9
Thalamus	1.1 ± 1.7	-2.7 ± 1.3*	3.8 ± 1.7*
Ventricular System			
All Ventricles	-5.3 ± 1.7*	6.8 ± 3.4*	12.1 ± 2.5*
Lateral Ventricles	-5.0 ± 2.0*	7.5 ± 3.5*	12.5 ± 2.7*
Third and Fourth Ventricles	-8.9 ± 4.3*	3.8 ± 3.9	12.8 ± 4.0*
Nonventricular Cerebrospinal Fluid	-6.2 ± 2.9*	6.0 ± 5.4*	12.2 ± 4.4*

ular volume and non-ventricular cerebral spinal fluid (between 5 and 6%). In direct contrast to this, EHS-DEH (vs. CON) increased volume of the ventricles and non-ventricular cerebral spinal fluid (by 6.0 - 6.8%), but also decreased volume of the cerebellum (by 0.7%), subcortical grey matter (by 1.1%), and thalamus (by 2.7%). In addition, EHS-DEH (vs. EHS) decreased volume of cortical white matter, subcortical gray matter, cerebellum, cerebellar gray matter, and corpus callosum.

Figure 3.2 provides the mean and individual data for selected anatomical sites to illustrate this dichotomy between trials. EHS consistently increased cerebral gray matter and thalamus volume (assumed fluid gain). EHS-DEH consistently decreased tissue volume (assumed fluid loss) concomitant with ventricular volume changes in the opposite direction (i.e., reduction with EHS, presumably as surrounding tissues gained fluid, and expansion

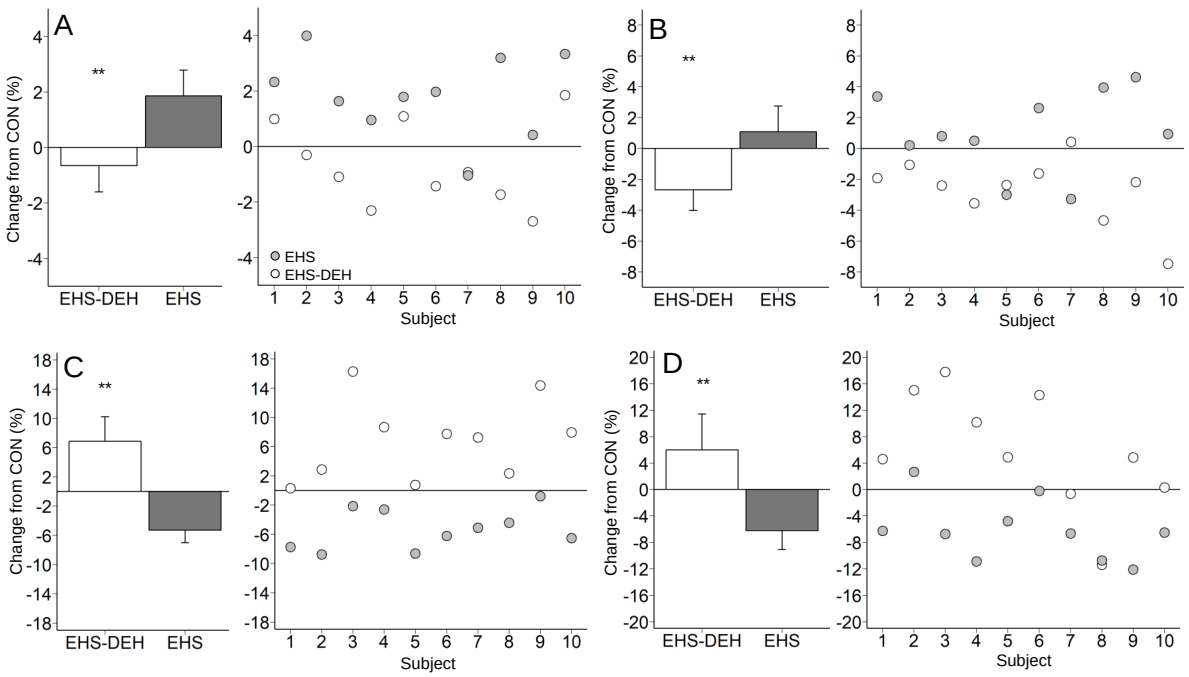

Figure 3.2: *Left panel A-D*: Mean (± 95% Confidence Interval) relative change (from resting control; CON) within cerebellar grey matter (A), thalamus (B), all ventricles (C), and non-ventricular cerebrospinal fluid (D), during exercise heat stress (EHS) and exercise heat stress coupled with dehydration (EHS-DEH). *Right panel within A-D*: Relative changes for each individual subject (n = 10) following EHS (filled circles) and EHS-DEH (open circles). * $p < 0.05$, ** $p < 0.01$ vs EHS

with EHS-DEH, as surrounding tissues crenate from fluid loss).

Figure 3.3A-D presents the relationship between changes in specific brain structures (thalamus, cerebellum) with their adjacent ventricles and change in POsm. Change in POsm was inversely correlated with changes in cerebellum (r = -0.61, p = 0.005), cerebellar grey matter (r = -0.63, p = 0.003), and thalamus (r = -0.45, p = 0.04) volumes but directly associated with changes in total ventricular (r = 0.74, p = 0.0002) and non-ventricular cerebrospinal fluid volumes (r = 0.70, p = 0.0006). Moreover, lateral ventricle (r = -0.41) and fourth ventricle (r = -0.67) expansion were significantly ($p < 0.05$) associated with reductions in thalamus and cerebellum volume, respectively. VMPT performance was not associated with changes in brain structures or POsm ($p > 0.05$). VMPT performance was not associated with changes in brain structures or POsm ($p > 0.05$). Figure ??????E-F present a scatter plot illustrating the relationship between changes in thalamus

and cerebellum volume and changes in VMPT accuracy.

3.4.4 Brain Function via BOLD During VMPT

Figure 3.4 presents BOLD responses obtained from a data set of n = 10 during CON to illustrate the task-dependent neural resource requirements during the entire 20-min VMPT (combining regular and irregular variations). Elevated BOLD responses were observed for the following brain areas: left sensorimotor, bilateral supplementary motor, bilateral thalamus, bilateral putamen, bilateral caudate, right frontal pole, bilateral middle and superior frontal gyri, supramarginal gyrus, angular gyrus, left frontal, central, and right operculum, and bilateral insula.

BOLD response contrasts for the entire 20-min are presented in Table 3.2 and Figure 3.5. No differences in BOLD responses were observed between EHS and CON. However, EHS-DEH elicited two elevated BOLD clusters ($p < 0.05$) compared to CON. The first cluster was located within the bilateral thalamus, bilateral caudate, right putamen, right pallidum, right parietal lobe, right insula, and anterior cingulate cortex while the second cluster was located within the left temporal lobe, left frontal lobe, left putamen, left amygdala, left hippocampus, and left insular cortex. EHS-DEH also elevated BOLD responses ($p < 0.05$) vs. EHS within the bilateral frontal lobe, bilateral thalamus, bilateral caudate, left central operculum, left insular cortex, anterior cingulate cortex, and supplementary motor area (Table 3.2, Figure 3.5).

No differences in BOLD responses were observed among trials during irregular paced intervals (VMPTi). During the regular timed intervals (VMPTr), no significant differences in BOLD responses were observed between EHS and CON; however, EHS-DEH elicited elevated BOLD responses vs. CON ($p < 0.05$) within the bilateral thalamus/basal ganglia, hippocampus, insula, left amygdala, and posterior cingulate (Table 3.3, Figure 3.6). In addition, EHS-DEH elevated BOLD responses vs. EHS ($p < 0.05$) within the right caudate, frontal lobe, right temporal lobe, and anterior cingulate gyrus (Table 3.3, Figure 3.6).

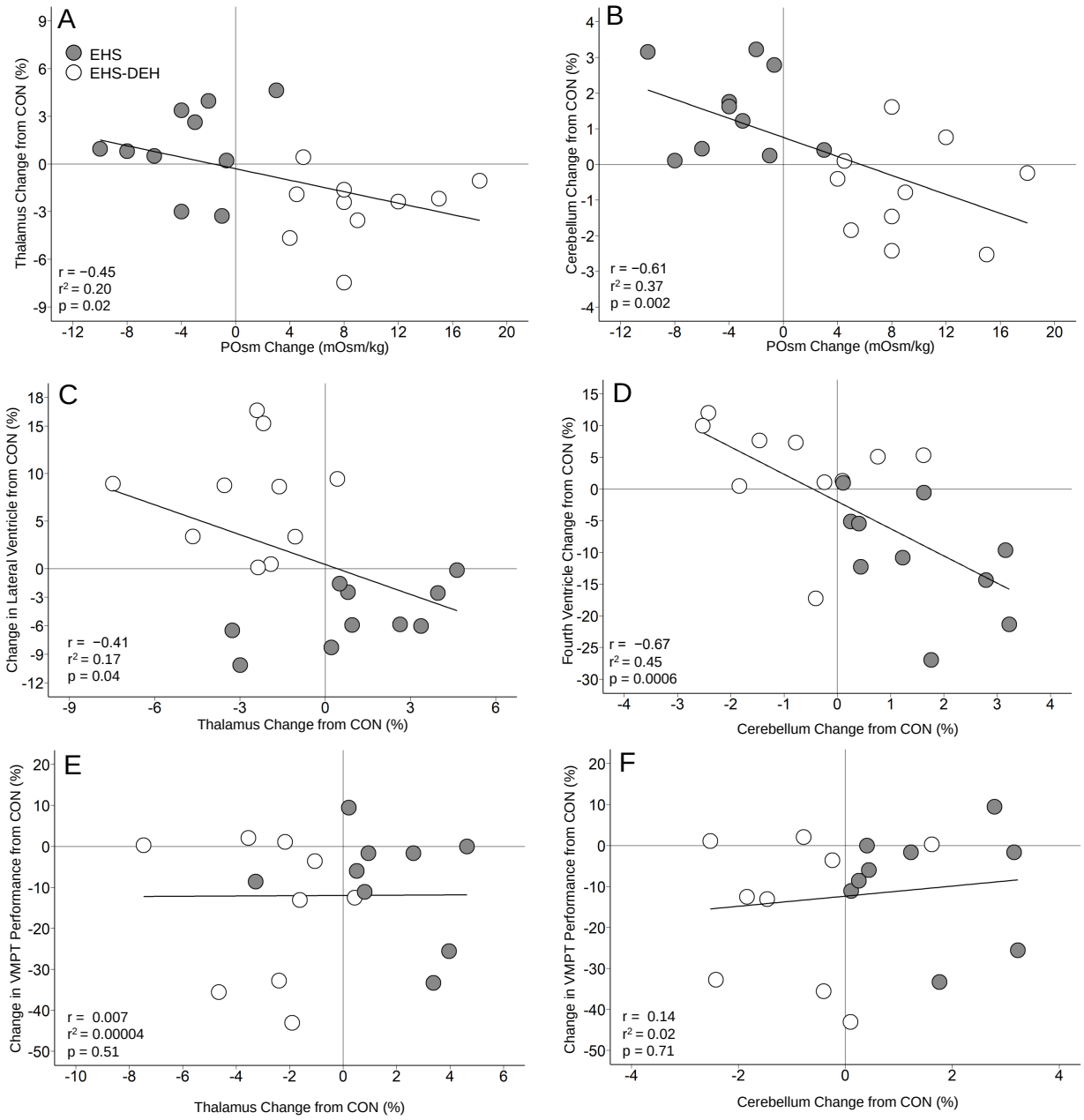

Figure 3.3: Association between changes in plasma osmolality (from pre-exercise baseline) and brain structure changes (relative to resting control; CON) in thalamus (A) and cerebellum (B). Associations between relative changes (from CON) in the thalamus (C) and cerebellum (D) with interfacing ventricular structure. Associations between relative changes (from CON) in the thalamus (E) and cerebellum (F) with mean change (from CON) in visuomotor task (VMPT) accuracy. Circles indicate individual data (n = 10) by trial: exercise heat stress with fluid replacement (EHS, closed circles) and exercise-heat stress without fluid replacement (EHS-DEH, open circles).

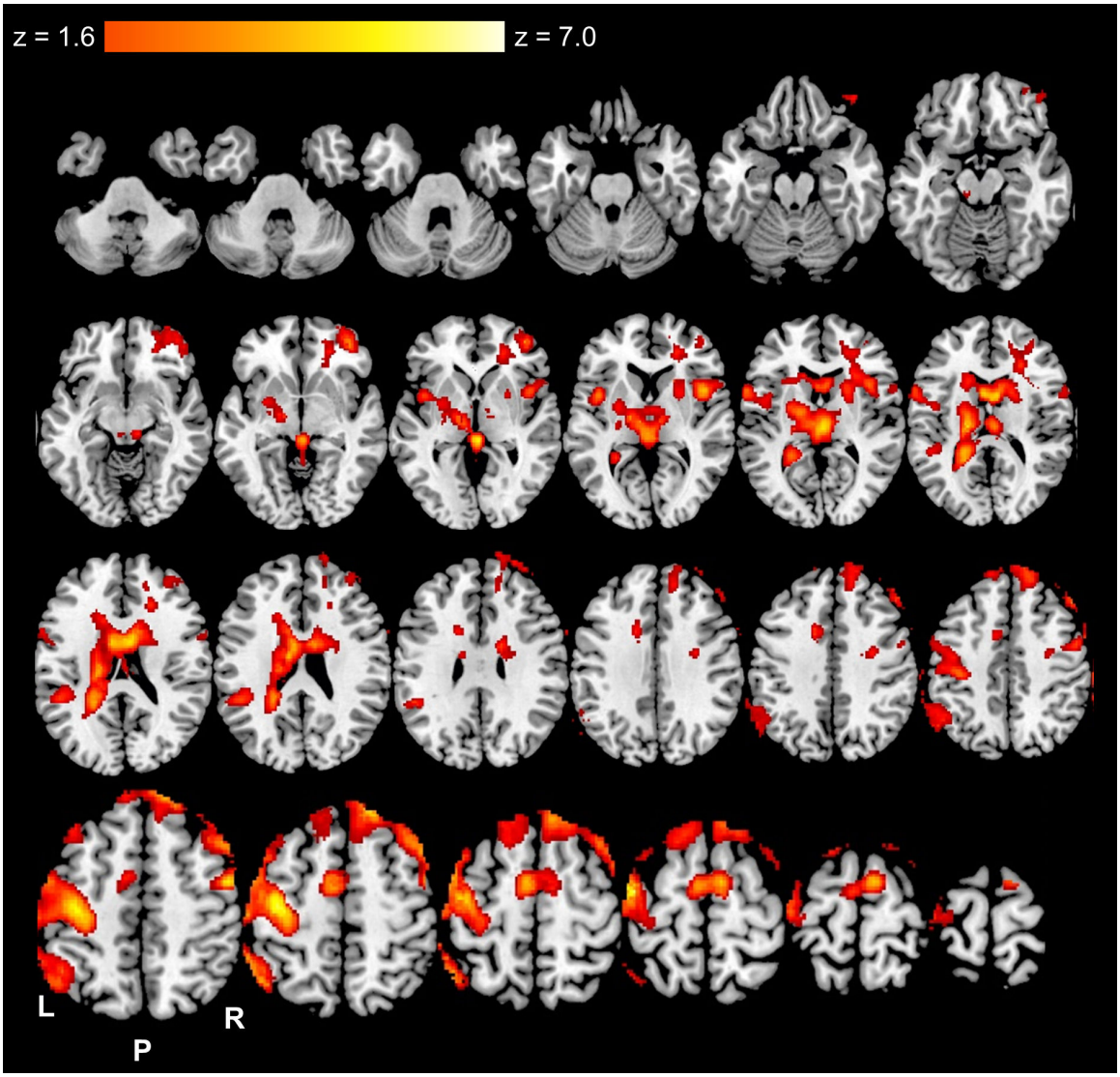

Figure 3.4: Axial slices of significantly ($Z \geq 1.6$ with cluster correction of $p < 0.05$) elevated blood oxygen level dependent (BOLD) responses throughout the entire visuomotor pacing task during the resting control (CON) trial (n = 10). Color gradient indicates level of elevated BOLD responses.

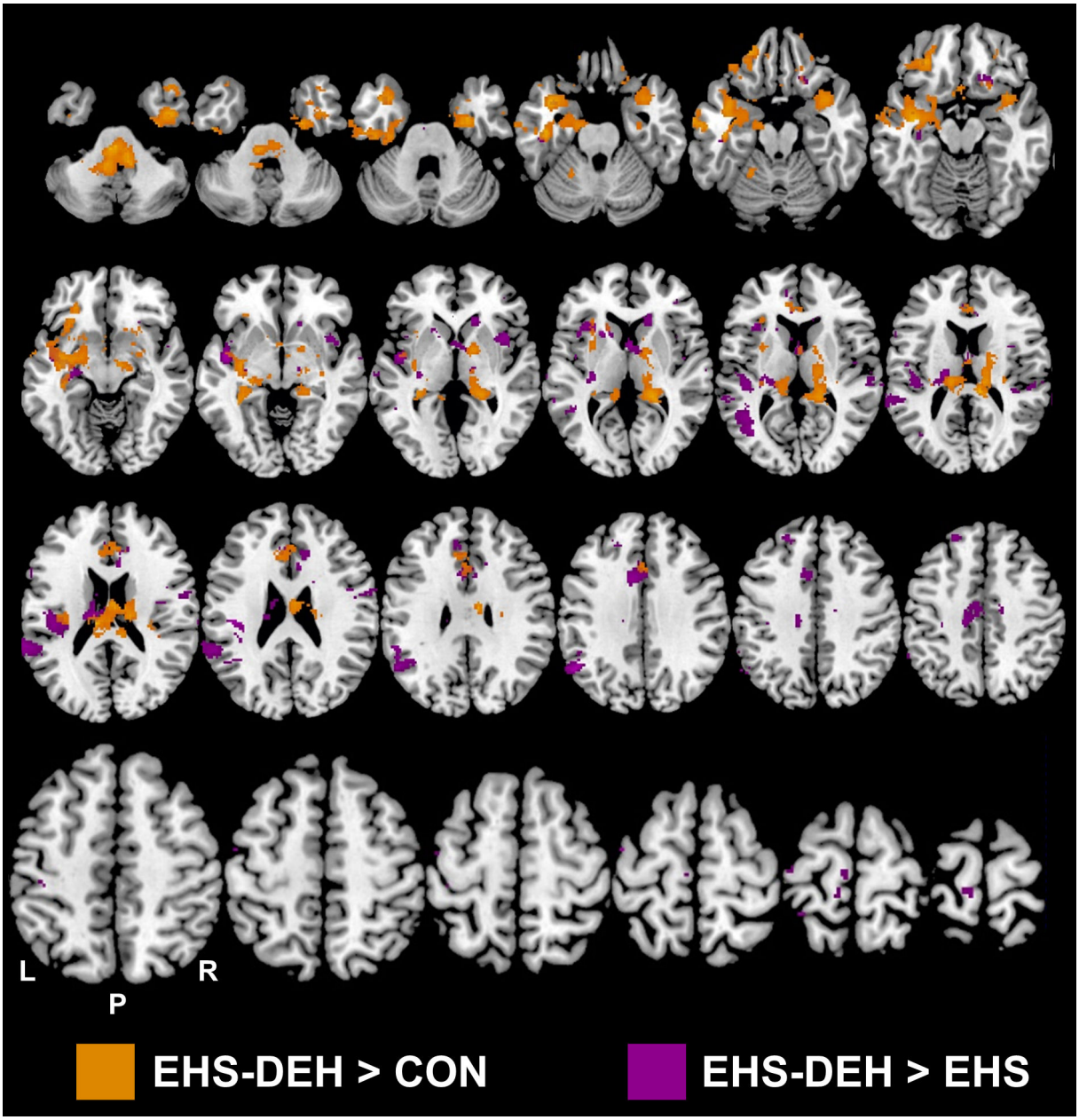

Figure 3.5: Significantly elevated ($Z \geq 2.5$ with cluster correction of $p < 0.01$) blood oxygen level dependent (BOLD) responses for the entire visuomotor pacing task during exercise heat stress with dehydration (EHS-DEH) compared to resting control (CON) and exercise heat stress without dehydration (EHS; n = 10). Areas of color indicate locations where EHS-DEH elicited greater BOLD responses compared to CON or EHS. No differences were observed between CON and EHS ($p > 0.05$)

Table 3.2: Significantly elevated ($Z \geq 2.5$) BOLD responses observed throughout the entire visuomotor pacing task for exercise heat stress with dehydration (EHS-DEH) compared to resting control (CON) and exercise heat stress without dehydration (EHS; n = 10). Cluster peaks are presented in MNI152 coordinates. Italicized areas indicate task-specific areas as identified during CON.

	Regions in Cluster	Hemisphere
EHS vs. CON	No significant clusters	
EHS-DEH > CON		
Cluster 1 Peak: 22, -40, 6	*Caudate*	*Right*
Voxels: 2105	*Caudate*	*Left*
Peak Z: 4.4	Cingulate Gyrus, Anterior	Right
	Cingulate Gyrus, Posterior	Right
	Hippocampus	Right
	Insular Cortex	*Right*
	Pallidum	Right
	Parietal Lobe, Operculum Cortex	Right
	Putamen	*Right*
	Subcallosal Cortex	
	Thalamus	*Right*
	Thalamus	*Left*
Cluster 2 Peak: -38, -8, -12	Amygdala	Left
Voxels: 2505	Frontal Lobe, Inferior Gyrus	Left
Peak Z: 4.2	*Frontal Lobe, Operculum Cortex*	*Left*
	Frontal Lobe, Orbital Cortex	Left
	Frontal Lobe, Pole	Left
	Insular Cortex	*Left*
	Putamen	*Left*
	Temporal Lobe, Fusiform Cortex	Left
	Temporal Lobe, Inferior Gyrus	Left
	Temporal Lobe, Pole	Left
	Temporal Lobe, Planum Polare	Left
EHS-DEH > EHS		
Cluster Peak: -20, 16, 4	*Caudate*	*Right*
Voxels: 1905	*Caudate*	*Left*
Peak Z: 4.1	*Central Operculum Cortex*	*Left*
	Cingulate Gyrus, Anterior	
	Cingulate Gyrus, Posterior	
	Frontal Lobe, Pole	Left
	Frontal Lobe, Middle Frontal Gyrus	*Right*
	Frontal Lobe, Middle Frontal Gyrus	*Left*
	Frontal Lobe, Superior Frontal Gyrus	*Left*
	Insular Cortex	*Left*
	Paracingulate Gyrus	Left
	Putamen	*Left*
	Supplementary Motor Area	
	Thalamus	*Right*
	Thalamus	*Left*

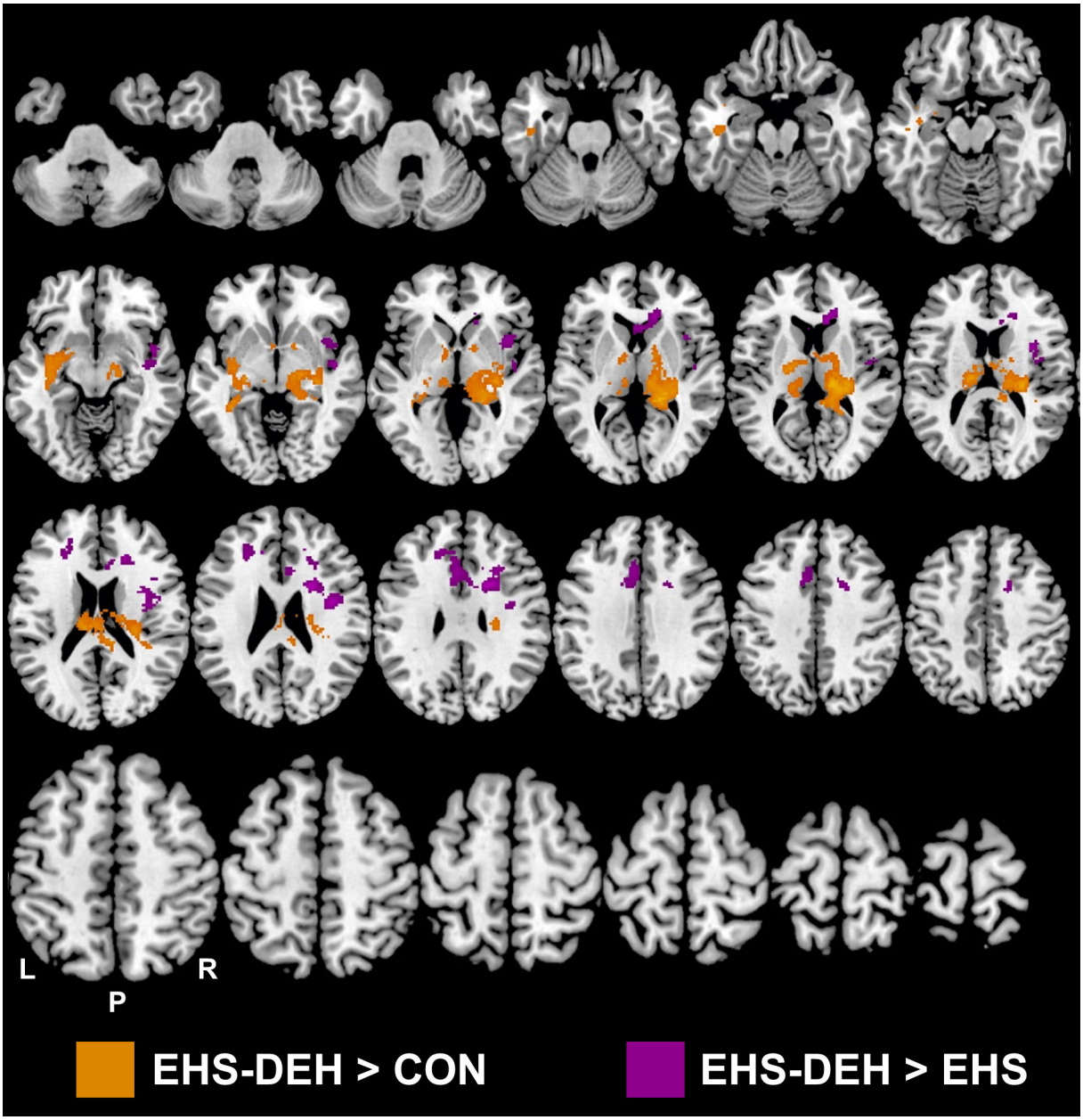

Figure 3.6: Significantly elevated ($Z \geq 2.5$) BOLD responses observed during the regularly paced stimuli within the visuomotor pacing task (VMPTr) for exercise heat stress with dehydration (EHS-DEH) compared to resting control (CON) and exercise heat stress without dehydration (EHS; n = 10). Cluster peaks are presented in MNI152 coordinates. Italicized areas indicate task-specific areas as identified during CON.

Table 3.3: Significantly elevated ($Z \geq 2.5$ with cluster correction of $p < 0.05$) blood oxygen level dependent (BOLD) responses during regularly paced stimuli within the visuomotor pacing task (VMPTr) for exercise heat stress with dehydration (EHS-DEH) compared to resting control (CON) and exercise heat stress without dehydration (EHS; n = 10). Areas of color indicate locations where EHS-DEH elicited greater BOLD responses compared to CON or EHS.

	Regions in Cluster	Hemisphere
EHS vs. CON	No significant clusters	
EHS-DEH > CON		
Cluster Peak: 16, -24, 10	Amygdala	Left
Voxels: 3252	Cingulate Gyrus, Posterior	
Peak Z: 4.7	Hippocampus	Right
	Hippocampus	Left
	Insular Cortex	Right
	Insular Cortex	Left
	Pallidum	Right
	Parahippocampal Gyrus	Left
	Parietal Lobe, Operculum Cortex	Right
	Putamen	*Left*
	Putamen	*Right*
	Thalamus	*Left*
	Thalamus	*Right*
EHS-DEH > EHS		
Cluster Peak: 24, 10, 26	Anterior Cingulate Gyrus	
Voxels: 1646	*Caudate*	*Right*
Peak Z: 4.4	*Central Operculum Cortex*	*Right*
	Cingulate Gyrus, Anterior	
	Frontal Lobe, Middle Gyrus	*Right*
	Frontal Lobe, Superior Gyrus	
	Frontal Lobe, Pole	
	Insular Cortex	*Right*
	Paracingulate Gyrus	
	Subcallosal Cortex	Right
	Termporal Lobe, Planum Polare	Right
	Temporal Lobe, Superior Gyrus	Right

3.5 Discussion

Dehydration is a common perturbation in occupational, military, sports, and elderly populations. Early research observed that dehydration alters multiple cognitive-motor domains [70, 71], however, many subsequent studies have not supported this position [14, 13, 127]. Our data uniquely focused on visuomotor performance while simultaneously examining changes in brain structure and function. Previous research has not focused on visuomotor timing, a core tenant of visuomotor function [151]. I designed the VMPT to mirror occupational, military tasks requiring prolonged visuomotor output at 1 Hz, the frequency at which the human motor control operates [151]. In addition, I controlled for potentially confounding factors in previous studies such as prior exercise-heat stress and body water deficits insufficient to induce physiological compensation [50] and an extended cool-down period to mitigate residual effects of exercise and heat exposure.

A major finding was that prior exercise-heat stress with and without dehydration each impaired visuomotor performance (by $\sim$8% for exercise-heat stress and $\sim$15% when coupled with dehydration), and degradation appearing early (within first five minutes). Thus, in contrast to our hypothesis, dehydration increased brain activation during the visuomotor task but resulted in marked impairments. That a simple visuomotor task was impaired runs counter to previous work [77] suggesting tasks requiring executive functioning are more likely impacted by dehydration. This belief persists given the highly-integrative nature of executive function tasks [46] which impart a greater cognitive-motor stress compared to the single stimulus-response pairing in the VMPT. Our visuomotor performance impairments are congruent with some [70, 74], but not all [75], studies examining fine control of motor coordination at similar body water deficits ($\sim$3% BM loss) following a post-exercise recovery. However, these previous studies assessed motor coordination utilizing either rudimentary (non-computerized) [70] or brief ($\sim$3 min) tasks with few stimuli which, following similar protocols by the same research group, have both observed [74] and failed to find

[75] impairments following dehydration. Our findings contrast with another study reporting improved fine motor performance (maximal finger tapping frequency in 10s) following dehydration following a soccer match in the desert [149], although the specific water deficit incurred was not specified.

I acknowledge that degraded visuomotor performance was not consistent across all subjects. The reason several (n = 4) remained resilient in maintaining accuracy across the trials (and despite dehydration) are unclear other than the observation that most tended to have high proficiency during the VMPT and may have sufficient visuomotor reserve. When examining potential confounders (e.g., self-reported fitness status, gender, month tested), no individual factors appear to account for subject resilience. The VMPT, a monotonous task containing only one stimulus, is characteristic of tasks which stress attentional capacity [165]. Although dehydration per se did not alter the rate of performance decline over time ($\sim$9% over the 20 min across all trials), I cannot exclude the possibility that performance impairments following both exercise-heat stress trials are partially explained by a diminished capacity of visual-spatial resources.

Another major finding was elevated neural activity (i.e., increased BOLD responses) when performing a visuomotor task following dehydration with exercise-heat stress but not after exercise-heat stress alone. Thus, our findings contradict a prior study with modest dehydration [46] which reported elevated neural activity in fronto-parietal areas without executive function impairments, suggesting elevated brain activations represented neural inefficiencies. Elevated neural activity following dehydration in the current study occurred in anatomical areas previously observed during right index finger tapping: the left thalamus, left putamen, and left caudate [166], suggesting that greater brain activation was required within task-specific areas. Additionally, dehydration also elicited greater ipsilateral neural activity (i.e., right thalamus/basal ganglia). During unimanual finger tapping, bilateral neural activity potentially indicates: i) greater neural demands during time keeping, planning, and/or execution of finger tapping [167, 166] or ii) greater attentional resources for task

execution [168].

A surprising finding was that dehydration elevated neural activity in cortical areas not observed under resting, control conditions such as the hippocampus, inferior, middle, and superior temporal lobes. I believe these findings may represent altered visual processing following dehydration. The inferior temporal lobe integrates neural input from the primary visual cortex to encode the perception of objects (i.e., ventral visual stream) [169]. While the dorsal stream, located within the parietal lobe [170], is responsible for the motor aspects of vision [169], ventral stream activation provides necessary object information for motor-specific visual processing [171]. Alternatively, the medial temporal lobe may integrate information between the ventral and dorsal processing streams [172], suggesting dehydration increases neural resource requirement in the visual processes leading to planning and execution of visuomotor movements.

In contrast, I believe that elevated neural activity in this study could indicate additive neural demands of VMPT task completion and homeostasis maintenance, as evidenced by elevated brain activations within brain areas (e.g., anterior cingulate and superior temporal gyrus) known to be involved with signaling thirst [146]. Furthermore, a right-lateralized network involving both the anterior cingulate and thalamus mediates attentional resources [165], which, when combined with the progressive decline in accuracy, could also partially explain increased ipsilateral neural activity following dehydration. Because the elevated neural activity in the current study include areas responsible for a wide array of neurological functions (e.g., cognitive-motor function, thirst response, attentional vigilance) [92], I propose that elevated brain activation following dehydration elicited by exercise-heat stress do not have a sole origin, but result from a confluence of sources.

Our study corroborates others observing that dehydration does not reduce total brain volume [45, 46, 44, 47], but provides novel information regarding changes to brain structures, including the ventricles. Similar to our data, two previous studies observed lateral ventricles expansion following dehydration [46, 45], however, others reported either no

change [43, 49, 47] or decreases in volume [44]. Various mechanisms have been presented to explain changes to brain ventricular volumes following dehydration such as: i) ventricular shrinkage as a consequence of overall body fluid losses [44] or ii) ventricular expansion via an ex vacuo mechanism resulting from osmotic gradients drawing fluid out of the intracellular spaces [45].

Our findings support the existence of an ex vacuo expansion of the ventricular system during dehydration where elevated POsm results from the loss of hypotonic sweat. Increased POsm also elevates osmolality of cerebrospinal fluid [173, 174], as the choroid plexus may secrete a fluid isotonic to blood plasma [175]. Hypertonic cerebrospinal fluid decreases choroid plexus bulk flow (i.e., production of cerebrospinal fluid), potentially to maintain total brain volume, as evidenced by shrinking brain volume only occurring with severely elevated cerebrospinal fluid osmolality (increase of 45 mOsm/kg) [175]. This mechanism likely explains why total brain volume was unchanged following dehydration but ventricular volume and non-ventricular cerebrospinal fluid were expanded. Further support of this mechanism is presented with the changes in periventricular structures inversely associated with both POsm and ventricular volumes, suggesting transfer of fluid between the two compartments.

During exercise in the heat with water replacement to match 100% sweat loss, our subjects experienced a small decrease ($\sim$3 mOsm/kg) in plasma osmolality with the unexpected finding of opposing changes to brain structures (e.g., ventricular constriction, periventricular tissue swelling) compared to exercise-heat stress with dehydration. Hypotonic fluid perfusing the ventricular system will increase choroid plexus bulk flow, decreasing cerebrospinal fluid osmolality and expanding cortical grey and white matter [175], consistent with our finding. Given the potential dilution effect (drop in plasma osmolality when water ingestion completely replaces sweat losses) similar to other studies [176, 177], the cerebrospinal fluid osmolality may decrease in parallel. Therefore, during exercise-heat stress, when sweat is replaced by electrolyte-free water, brain structure volume regulation

may begin to induce brain swelling, similar to effects of hyponatremia [178]. However, to our knowledge, previous research has not shown such relatively small plasma osmolality changes ($\sim$3 mOsm/kg) to be associated with changes in brain structure.

Although both exercise-heat stress with and without water replacement altered brain structure volume, no clear association with visuomotor performance was observed. One might hypothesize that net tissue volume changes (in either direction), mediated by osmolality perturbations (as shown in Figure 3), could explain performance impairment since both cerebral edema (e.g., hyponatremia) and dehydration symptoms include deteriorated mental status [1, 179]. However, this hypothesis is not supported by Figure 3.3 (E-F), as some individuals with the largest thalamus expansions and contractions demonstrated sustained visuomotor accuracy.

3.6 Conclusion

In summary, our study is the first to simultaneously assess brain activation using fMRI while performing a visuomotor task at body water deficits that require homeostatic responses [50]. This study made the following novel observations: 1) cognitive-motor performance is impaired with exercise-heat stress and further exacerbated by dehydration; 2) Several brain structure volumes are sensitive to both increases and modest decreases in plasma osmolality; and 3) additional neural activity was observed following dehydration during a simple finger tapping task. These observations suggest dehydration may elicit fundamental impairments in the visuomotor system which could potentially impact military and occupation-specific tasks requiring a motor output.

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CHAPTER 4

AIM 3: EVALUATE THE IMPACT OF DEHYDRATION ON MOTOR PLANNING

4.1 Abstract

This study determined the impact of dehydration on motor planning as evaluated by performance and brain activations using electroencephalography (EEG) during a visuomotor task. Ten males were assessed following exercise-heat stress either with (EHS) and without water replacement ($\sim$3% body mass loss; EHS-DEH) compared to rest (CON). Performance (reaction time, accuracy) during a bimanual probabilistic choice reaction time task (PCRT) incorporating dominant (frequent) and non-dominant (infrequent) responses was evaluated concurrent with function by visual evoked potentials (contingent negative variation, N1, N2). PCRT reaction time was not different across trials (p = 0.40). EHS-DEH (67.3 $\pm$ 14.1%) reduced PCRT accuracy during non-dominant responses vs CON (83.7 $\pm$ 5.8 %; p = 0.04) but not compared to EHS (74.6 $\pm$ 11.0 %; p = 0.18). No differences were observed during dominant responses ($p > 0.05$) across trials. EHS-DEH increased N1 amplitude in the occipital electrodes (385.2 $\pm$ 141.3 uV*ms) compared to CON (241.8 $\pm$ 168.6 uV*ms; p = 0.001) but not EHS (300.3 $\pm$ 171.1 uV*ms; p = 0.60); EHS and CON were not different (p = 0.60). No differences were observed among trials for the contingent negative variation or N2 ($p > 0.05$). EHS-DEH (6.4 $\pm$ 5.0, 11.8 $\pm$ 5.0) also elicited greater levels of perceived effort compared to CON (3.7 $\pm$ 2.4; p = 0.03) and frustration compared to EHS (7.5 $\pm$ 5.1; p = 0.0004). N1 amplitude was associated (p = 0.006) with non-dominant side PCRT accuracy (r = 0.34) and rating of physical demand (p = 0.03). Dehydration elicits reduced well-being, greater perceived mental workload, increased N1 amplitude, and impaired accuracy during motor planning. Dehydration impairs visuomotor performance by adversely impacting both motor execution (Aim 2) and planning (Aim 3).

4.2 Introduction

Dehydration is believed to impair cognitive-motor performance [71, 70, 148], although this has not been consistently demonstrated [14, 13, 127]. Furthermore, if and how dehydration alters specific cognitive-motor domains (e.g., executive function, information processing, memory, reaction time), the visuomotor system (motor planning, motor execution), and sensory systems (e.g., vision, auditory) is not well understood. The meta-analysis in Aim 1 indicated that executive functions were the cognitive-motor domain most likely affected by dehydration; whereas, information processing and reaction time were not. There was insufficient data to determine whether dehydration impaired visuomotor performance, despite the fact that most cognitive-motor tasks in the meta-analysis utilized visual stimuli. An observation from the meta-analysis was that studies examining visuomotor performance utilized short test durations ($<$ 5 min) [74, 70, 75] and therefore minimized the importance of attention, one of the executive functions. In addition, the short duration studies generated a limited number of observations thus minimizing statistical power.

Aim 2 was designed to assess visuomotor performance while also requiring prolonged attention (task duration of 20 min). We selected a well-accepted test of visuomotor performance, finger tapping [92], in order to focus on motor execution since only one stimulus-response combination was possible [180, 181, 182]. Aim 2 demonstrated that dehydration impaired visuomotor performance, with decrements predominantly attributed to motor execution despite greater brain activations during the task and volume changes in motor areas (e.g., thalamus). Given that movements require both planning and execution, we determined that the effects of dehydration on motor planning required further investigation. Thus, a challenge for Aim 3 was to customize a visuomotor test that sufficiently emphasized motor planning with similar execution demands (i.e., prolonged rhythmic button pressing) to determine if dehydration preferentially impacted either of these components.

To construct a prolonged (i.e., $>$20 min) visuomotor task with sufficient motor planning

demands, we evaluated the drift-diffusion model [96]. The drift-diffusion model postulates that, after a stimulus is observed, an individual accumulates evidence resulting in the selection and execution of a movement [96]. Evidence accumulation (i.e., motor planning) is required to process and respond to each stimulus [96]. Instituting multiple stimuli in the Aim 3 task will therefore increase motor planning demands by requiring selection of a response. Secondly, neural processes associated with motor planning are affected by previous expectations. Frontal and parietal neural activity are shown to increase as a result of being biased towards one response, consistent with altered motor planning [125]. Therefore, in addition to multiple stimuli, the Aim 3 task will alter the stimuli presentation rates to examine the specific effects of dehydration on motor planning, motor execution, and attention.

To assess brain activity during the visuomotor task, Aim 3 will employ analysis of visual-evoked potentials (VEP), specifically the contingent negative variation (CNV), N1, and N2, as recorded with electroencephalography (EEG). I will examine frontoparietal brain areas previously observed to be involved in motor planning [97, 183, 120] along with cortical areas involved with vision (occipital lobe). The CNV occurs in response to a warning stimulus [119, 118] with the early component related to processing the stimulus presence and a later component related to preparing for a motor response [119]. CNV amplitude appears modulated by learning the task demands, with lower amplitudes observed when individuals become acclimated to the task (i.e., decreased motor planning demands) [119, 184]. The two other VEP components of interest, the N1 (negative deflection occurring 100 ms post stimulus onset) and N2 (200 ms post onset) [185] will be examined. These components are involved in visual processing and spatial attention (N1) and stimulus categorization (N2) [113]. I hypothesize that, similar to Aim 2, dehydration will impair accuracy during the motor planning task but not alter reaction time. Secondly, greater N1 and N2 amplitudes likely indicate either greater arousal (N1) or stimulus categorization demands (N2) [113], and therefore I hypothesize that VEP amplitude (i.e., brain activation)

will be greater for the N1 and N2 components following DEH. I also hypothesize that dehydration will decrease CNV amplitude, indicating lessened preparatory neural processing [119].

4.3 Methods

4.3.1 Participants

Ten right-handed healthy, aerobically fit males (age: 22.4 $\pm$ 2.5 y, body mass: 63.5 $\pm$ 5.9 kg, body fat: 14.7 $\pm$ 3.2%, maximal oxygen uptake: 59.0 $\pm$ 4.7 mL/kg/min) participated in the study. All subjects served as their own control and completed each condition. All subjects gave written, informed consent as approved by the Georgia Institute of Technology Institutional Review Board.

4.3.2 Experimental Design

Subjects completed two preliminary sessions and three experimental trials, all within $\sim$3 weeks. Before all sessions, subjects were instructed to consume $\sim$1 L of fluids the previous evening, abstain from alcohol for 12 h previous, and arrive following an overnight fast. Two preliminary sessions were instituted to establish baseline body mass (BM), plasma osmolality (POsm; $\leq$290 mOsm/kg), and urine specific gravity (USG; $\leq$1.020; *ATAGO USA, Bellevue, WA*) [50].

During one preliminary session, a graded exercise test was completed by subjects to measure aerobic exercise capacity / peak oxygen uptake. Before each graded exercise test, the metabolic cart (*Parvomedics, Sandy, UT*) was calibrated using a 3-L volume syringe and gases of known concentrations (16% O2, 4% CO2). The graded exercise test consisted of a modified Bruce protocol [186] starting at an initial brisk walking pace ($\sim$3.5 mph, 0% grade) with subsequent two-minute stages with grade increases of 2.5% until 7.5% was reached. At 7.5%, running speed increased 1 mph every two minutes until volitional fatigue (i.e., dictated by participant). All participants were required to achieve $>$60th percentile of

aerobic fitness (45 ml/kg/min) [186] to participate in the experimental trials. During the preliminary sessions subjects also practiced a shortened (5 min) version of the visuomotor task (described below) to limit any learning effects.

Following the preliminary sessions, subjects completed three experimental trials: control (CON; no exercise-heat stress), exercise-heat stress with water replacement (EHS), and exercise-heat stress with dehydration (EHS-DEH). The order of experimental trials was counterbalanced. The experimental trials were initiated in the morning ($\sim$0700) with first morning BM and POsm assessed and compared against preliminary session values to ensure adequate hydration status ($\leq$1% difference) [50]. Subjects then consumed a nutrition bar (250 kcal) and water (150 ml) before entering the hot (EHS, EHS-DEH; 45^{o}C, 15% RH) or temperate (CON; 22^{o}C, 30% RH) environment. During EHS and EHS-DEH, subjects completed 2.5 h of 45/15 min walk/rest intervals at a workload eliciting an initial heart rate of $\sim$110-120 bt/min ($\sim$3.5 mph, 5% grade). Exercise intensity and duration was equivalent during EHS and EHS-DEH. During EHS, subjects consumed a fluid volume equivalent to their sweat losses but no water was consumed during EHS-DEH. Following EHS and EHS-DEH, subjects were allowed to cool in a temperate environment ($\sim$30 min) prior to a final BM, blood glucose, and POsm measurement. Subjects were prepped for EEG (total recovery period: 45 min) and visuomotor testing. During CON, subjects reported under same baseline conditions and ingested the same meal but sat quietly for $\sim$1.5 h (in temperate environment) and did not engage in mentally stimulating activities before the EEG/visuomotor testing.

4.3.3 Physiological and Perceptual Measures

During all exercise trials, heart rate (HR) and rectal temperature (YSI, Yellow Springs, OH) were measured and did not exceed 90% of age-predicted HRmax (220-age) or 39.5^{o}C, respectively. The initial treadmill speed and grade elicited a heart rate of $\sim$120 bpm which corresponded to a relative exercise intensity of approximately $\sim$35% maximal aerobic ca-

pacity. Following the exercise-heat stress trials, all subjects had rectal temperature $<38.5^{o}$C before visuomotor testing. Blood samples were obtained by finger puncture and plasma osmolality measures as described previously using freeze point depression (*Osmette II, Precision Systems, Natick, MA*) [78]. Blood glucose was measured (*OneTouch UltraMini, LifeScan Inc., Wayne, PA*) post-exercise ($\sim$3 h after the meal) for EHS-DEH and EHS and $\sim$90 min after the meal for CON.

Rating of perceived exertion (RPE, Figure B.2) [187] was assessed at five min intervals during exercise. Before the EEG, subjects were asked to rate their thirst and well-being (10 cm visual analog scale [101]; Figure B.5). Following completion, subjects were asked to rate the mental exertion during the motor planning task using the NASA Task Load Index scale (NASA-TLX; Figure B.1) [188, 189]. The NASA-TLX asks subjects to assess their perceived workload of a given task using seven, 21-point scales. The domains of interest to quantify workload were: mental demand, physical demand, temporal demand, performance, effort, and frustration.

4.3.4 EEG Acquisition and Processing

Subjects were fitted with a standard 58-channel EEG cap (*Electrocap, Eaton, OH*) with a sampling rate of 1000 Hz *Synamps II, Neuroscan, Charlotte, NC, USA*) and referenced to the ear electrode. Scalp electrodes were prepared so that impedances were $<$ 5k. Brain activity was measured throughout the entire Probabilistic Choice Reaction Task (PCRT, described below).

The raw, continuous EEG was loaded into EEG lab [190] for subsequent processing. All data was first high-pass filtered at 0.5 Hz and low-pass filtered at 50 Hz before bad channels (kurtosis z value $>$5) were identified and rejected. Data was then epoched from 750 ms pre- to 750 ms post-stimulus onset with baseline correction from -750 to -650 ms. An independent component analysis (ICA) was then completed on each dataset with the *runica* algorithm from EEGLAB. Components for each dataset were examined to remove blink

and stereotypical artifacts based on visual inspection of scalp map localization, unusual spectral frequency patterns, and irregular VEP image activity. Data were then separated based on task lateralization (dominant, non-dominant stimuli). A Laplace transform was completed on the data according to previous research [191, 192, 193] before the subsequent trial/dominance analysis.

Subject datasets were loaded in the STUDY design and within-subject identifiers for trial (CON, EHS, EHS-DEH), and task lateralization (dominant, non-dominant) assigned. The VEP scalp map was then evaluated to identify electrodes of interest time intervals previously identified [113, 120]. The following time windows were used: -400 to -200 ms (Early CNV), -200 to 0 (Late CNV), 70 to 150 ms (N1), and 180 to 270 ms (N2). Within the given time intervals, head maps were consulted to determine areas of interest across trials. These electrode combinations included: N1 occipital electrodes (O1, OZ, O2), N2 in the centroparietal electrodes (CZ, C1, C2, PZA, C1P, C2P) and the early/late CNV component in the central electrode (CZ). Component amplitude values for each block were determined using the signed area amplitude method [113].

4.3.5 Probabilistic Choice Reaction Time Task (PCRT)

The PCRT is similar to serial choice reaction tasks [194] but featured prolonged (task duration $\sim$32 min) rhythmic (0.66 Hz) stimulus presentation (Figure 4.1). Choice reaction time tasks have previously been employed following dehydration [195, 13], although this is the first study to include a continuous, prolonged presentation of responses. The PCRT was chosen as it allows for the weighting of stimuli to manipulate motor planning demands [125, 196]. Upon viewing the stimulus (colored circle), subjects were instructed to press the right button (with right hand) if red or the left button (with left hand) if green. Responses were collected using a custom, two button keypad (*Arduino Uno, www.arduino.cc*). To press a button, subjects had to move index finger of left/right hand from a 'home base' to the button located 70 mm away in a reaching motion. Stimuli (750 ms display time)

were presented sequentially in-between fixation crosses (750 ms display time; Figure 4.1). Before starting the PCRT, the software (*PsychoPy v1.84.2*; *www.psychopy.org*) randomly selected the 'dominant' direction (right or left) and order of block presentation. Therefore, dominance side was not based on handedness. Each (n = 4) block consisted of 300 stimuli (lasting 7.5 min) and were only presented once, separated by a 45 s rest. Blocks altered in the dominant weighting (% of time prompted for the dominant response out of 300 stimuli; Table 4.1). Each stimulus was analyzed for reaction time (stimulus onset to button press) and accuracy (correct and timely response). Total test time was $\sim$ 32 minutes.

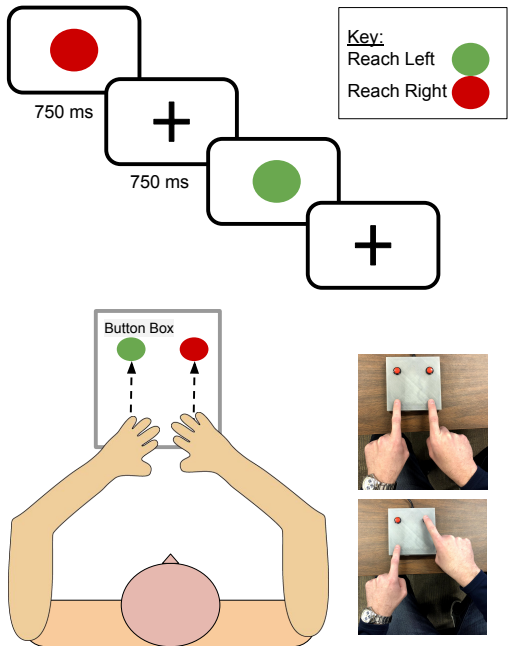


Figure 4.1: Schematic of Probabilistic Choice Reaction Task. Pictures indicate reaching motion starting at home base (top) and reach completion (bottom).

4.3.6 Statistical Analysis

All data were analyzed using a linear mixed effects model from the lme4 library within R (*cran.r-project.org/web/packages/lme4/*). Within the mixed effects model, trial (CON, EHS-DEH, EHS-EUH) was treated as a fixed (within) effect along with other subcomponents (e.g., NASA-TLX question) and subject treated as a random (within) effect. For the

Table 4.1: Experimental weighting of dominant and non-dominant side stimuli during the probabilistic choice reaction time task. Each block lasted 7.5 min.

Dominant Side		**Non-Dominant Side**		**Total**
%	**Number**	**%**	**Number**	
50%	150	**50%**	150	300
66%	198	**34%**	102	300
84%	252	**16%**	48	300
100%	300	**0%**	0	300

PCRT, only the 50%, 66%, and 84% blocks were analyzed by each side (dominance) as 100% did not have any non-dominant responses. The PCRT analysis treated trial, dominance weighting (50%, 66%, 84%), and dominant side (dominant, non-dominant) as fixed (within) effects. VEP amplitudes were examined using trial and side dominance fixed effects. If any significant main effects or interactions were observed, pairwise post-hoc comparisons were completed using the *lsmeans* package in R with Bonferroni-Holm post-hoc comparisons. Alpha level was set *a priori* at ≤ 0.05. Data is presented as means $\pm$ 95% confidence interval.

4.4 Results

4.4.1 Physiological and Perceptual Changes

As designed, baseline body mass was not ($p = 0.44$) significantly different among trials (CON: 73.7 $\pm$ 7.2; EHS-DEH: 73.2 $\pm$ 7.2; EHS: 73.4 $\pm$ 7.1 kg) and EHS-DEH elicited significantly greater ($p < 0.0001$) BM loss (3.1 $\pm$ 0.4%) compared to EHS (0.1 $\pm$ 0.6%). Baseline POsm (CON: 289.7 $\pm$ 2.5, EHS: 290.2 $\pm$ 2.1, EHS-DEH: 290.8 $\pm$ 2.0 mOsm/kg; $p > 0.05$) and USG (CON: 1.017 $\pm$ 0.002.5, EHS: 1.019 $\pm$ 0.004, EHS-DEH: 1.021 $\pm$ 0.004; $p = 0.15$) were similar among trials. However, EHS-DEH increased POsm (301.2 $\pm$ 2.0; $p < 0.0001$) from baseline which was higher ($p < 0.0001$) compared to EHS (286.0 $\pm$ 1.7 mOsm/kg) and CON. POsm following EHS was lower compared to baseline (by -4.2

Table 4.2: Mean ± 95% CI of Well-Being and Thirst Ratings (10 cm scale) [101] following resting control (CON), exercise-heat stress with fluid replacement (EHS), and exercise-heat stress without fluid replacement (EHS-DEH). # $p < 0.05$ vs. CON. * $p <$ vs. EHS.

	CON	EHS	EHS-DEH
Bad Taste in Mouth	1.4 ± 1.4	1.9 ± 1.6	5.1 ± 1.9 # *
Chalk Like Taste in Mouth	1.2 ± 1.6	1.3 ± 1.0	4.1 ± 2.1 # *
Dry Mouth	1.4 ± 1.6	3.1 ± 1.9	5.9 ± 2.2 # *
Feel Dizzy	0.9 ± 1.0	1.1 ± 0.6	2.1 ± 1.4 # *
Feel Light Headed	0.4 ± 0.3	2.2 ± 1.2	3.7 ± 1.6 # *
Feel Thirsty	1.9 ± 1.9	3.3 ± 2.2	6.6 ± 2.6 # *
Feel Tired	3.4 ± 1.4	4.5 ± 2.1	6.1 ± 1.0 # *
Feel Weak	0.9 ± 0.4	3.5 ± 1.5 #	4.3 ± 1.2 # *
Feel Weary	1.5 ± 1.4	2.3 ± 1.6	3.7 ± 1.2 # *
Mouth Irritated	0.3 ± 0.4	2.3 ± 1.8	3.5 ± 1.6 # *
Throat Feels Dry	1.3 ± 1.3	2.8 ± 1.8	6.1 ± 2.0 # *
Throat Feels Scratchy	0.2 ± 0.2	0.8 ± 0.5	3.8 ± 1.5 # *

± 1.7 mOsm/kg) and below that of the CON baseline value ($p = 0.0001$). Blood glucose was normal (> 4.0 mmol/L) in all subjects, with no differences among CON (5.3 ± 0.6), EHS (5.1 ± 0.3), or EHS-DEH (5.3 ± 0.6 mmol/L; $p = 0.41$) before beginning visuomotor testing. Table 4.2 presents the well-being and thirst questionnaire responses for CON, EHS, and EHS-DEH. EHS-DEH degraded well-being and increased thirst sensations compared to both CON and EHS ($p < 0.05$).

4.4.2 NASA Task Load Index

Figure 4.2 presents responses to the NASA-TLX perceived mental workload for the PCRT during CON, EHS, and EHS-DEH. In general, dehydration increased perceived task load during the PCRT while exercise-heat stress did not. For physical demand, a significant main effect for trial ($p = 0.02$) was observed. EHS-DEH (6.4 ± 5.0) elicited significantly greater perceived physical requirements compared to CON (3.7 ± 2.4, $p = 0.03$) but EHS (4.4 ± 5.0) was not different from either CON or EHS-DEH ($p > 0.05$). For perceived effort, a significant main effect for trial ($p = 0.01$) was observed. To perform the PCRT, EHS-DEH (13.5 ± 2.8) elicited greater perceived effort compared to both CON (9.4 ± 5.7;

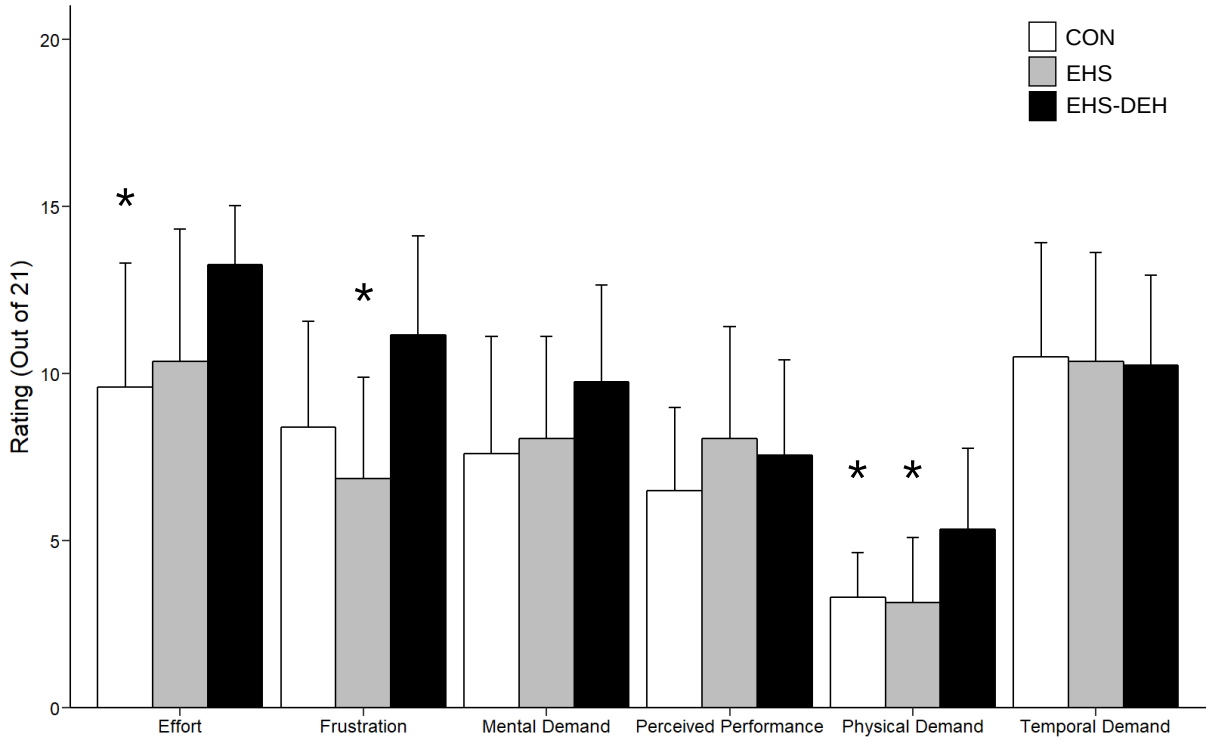


Figure 4.2: Mean ± 95% CI responses to the NASA-Task Load Index questionnaire (rating out of 21) for physical demand, frustration, temporal demand, perceived effort of task, mental demand, and perceived task performance following completion of a paced choice reaction task during resting control (CON), exercise-heat stress with water replacement (EHS), and exercise-heat stress without water replacement (EHS-DEH). * denotes significant ($p < 0.05$) difference from EHS-DEH.

p = 0.02) and EHS (9.8 ± 6.3; p = 0.03), but perceived effort was not different between CON and EHS (p = 0.78). For frustration, a significant main effect for trial (p = 0.005) was also observed. EHS-DEH (11.8 ± 5.0) elicited significantly greater frustration compared to EHS (7.5 ± 5.1, p = 0.004) but CON (4.4 ± 5.0) was not significantly different from either EHS-DEH or EHS ($p > 0.05$). No significant main effect for trial was observed for mental demand (CON: 7.0 ± 5.7; EHS-DEH: 9.3 ± 4.7, EHS: 7.5 ± 5.1; p = 0.07), temporal demand (CON 9.7 ± 5.9; EHS-DEH: 9.5 ± 4.7; EHS: 9.8 ± 5.3; p = 0.97), or self-rated performance (CON: 6.2 ± 3.9, EHS-DEH: 8.6 ± 5.7, EHS: 7.7 ± 5.2; p = 0.32).

4.4.3 Probabilistic Choice Reaction Task

Normal PCRT Task Demands (Control)

The PCRT responses based on percentage of dominant stimuli (50-100%) or '% weighting' and side dominance (dominant, non-dominant) are presented for the CON trial (Figure 4.3). PCRT reaction time (Figure 4.3A) was faster (decreased) during the dominant side responses (516.7 $\pm$ 37.6 ms) compared to non-dominant (560.0 $\pm$ 40.6 ms; $p < 0.0001$). The % weighting by side dominance interaction effect was significant ($p < 0.0001$). Figure 4.3A indicates at 50% weighting (equal number non-dominant and dominant stimuli), there was no difference in reaction time between the dominant and non-dominant sides ($p > 0.05$). As % weighting was increased towards the dominant side ($\geq$66%), dominant side reaction time (dashed line) became faster ($p < 0.0001$) than the 50% weighting, resulting in a difference ($p < 0.05$) compared to the non-dominant side (solid line). This is due to the fact that non-dominant side reaction time did not change ($p > 0.05$) across % weighting of the PCRT. Figure 4.3B presents the PCRT accuracy during CON by % weighting for the dominant and non-dominant sides. Overall accuracy did not differ significantly ($p = 0.07$) between the dominant (88.9 $\pm$ 6.4%) and non-dominant side (83.7 $\pm$ 5.8 %). There was also no % weighting by side dominance interaction ($p = 0.38$).

Trial Effects

Figure 4.4 presents the PCRT reaction time at each % weighting across trials (CON, EHS, EHS-DEH) for dominant (A) and non-dominant (B) sides. Overall reaction time for both sides combined was not different among trials (CON: 538.3 $\pm$ 37.7, EHS: 542.6 $\pm$ 39.2, EHS-DEH: 532.6 $\pm$ 39.2; $p = 0.40$). There were no significant interactions among trial, % weighting, or side dominance ($p > 0.05$; Table A.2).

Figure 4.5 presents the PCRT accuracy at each % weighting across trials (CON, EHS, EHS-DEH) for dominant (A) and non-dominant (B) sides. Overall accuracy for both sides

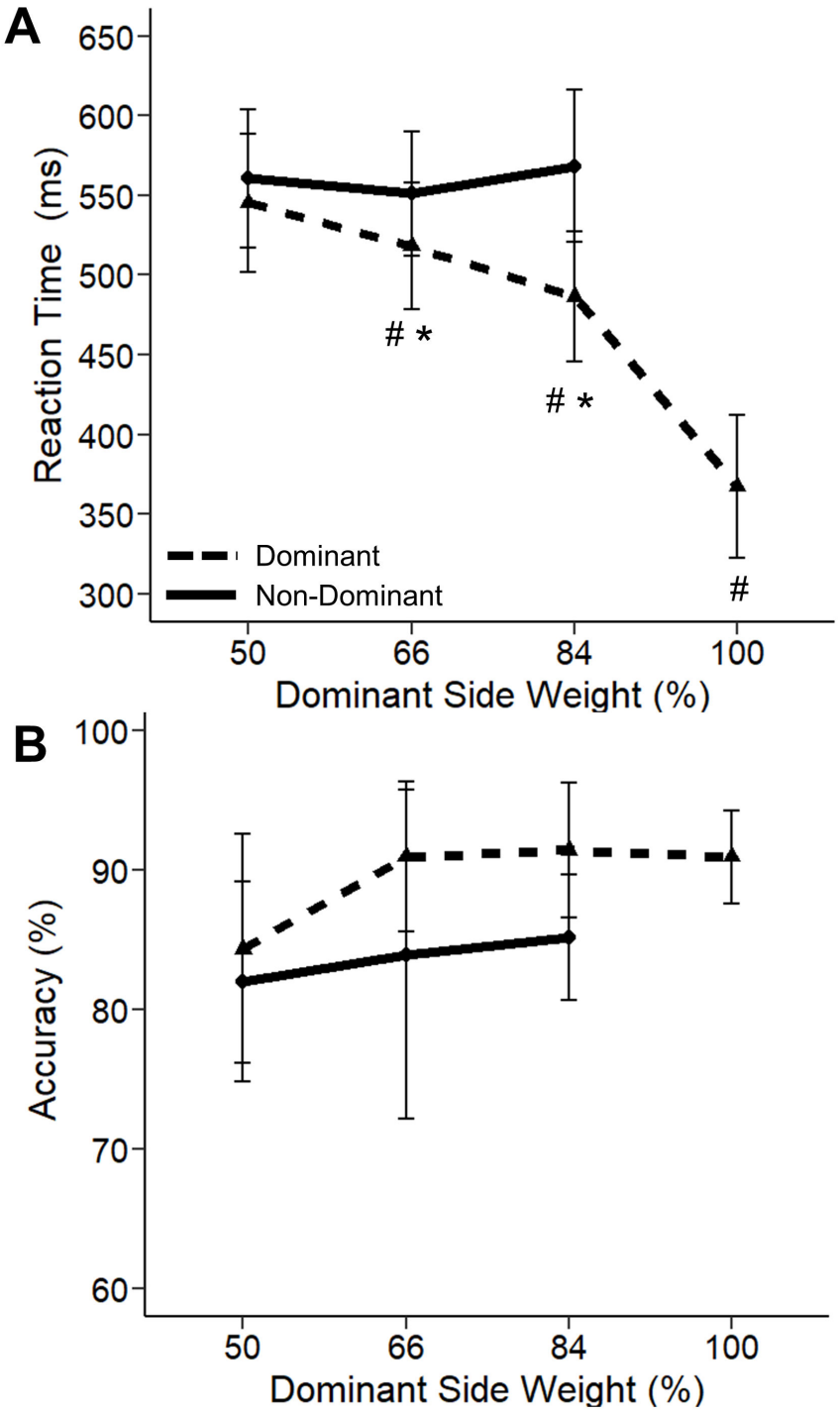


Figure 4.3: A: Mean $\pm$ 95% Confidence Interval probabilistic choice reaction task (PCRT) reaction time (A) and accuracy (B) during resting control dominant and non-dominant side responses. * indicates significant ($p < 0.05$) difference between dominant and non-dominant side at a given weight. # indicates significant ($p < 0.05$) difference from 50%.

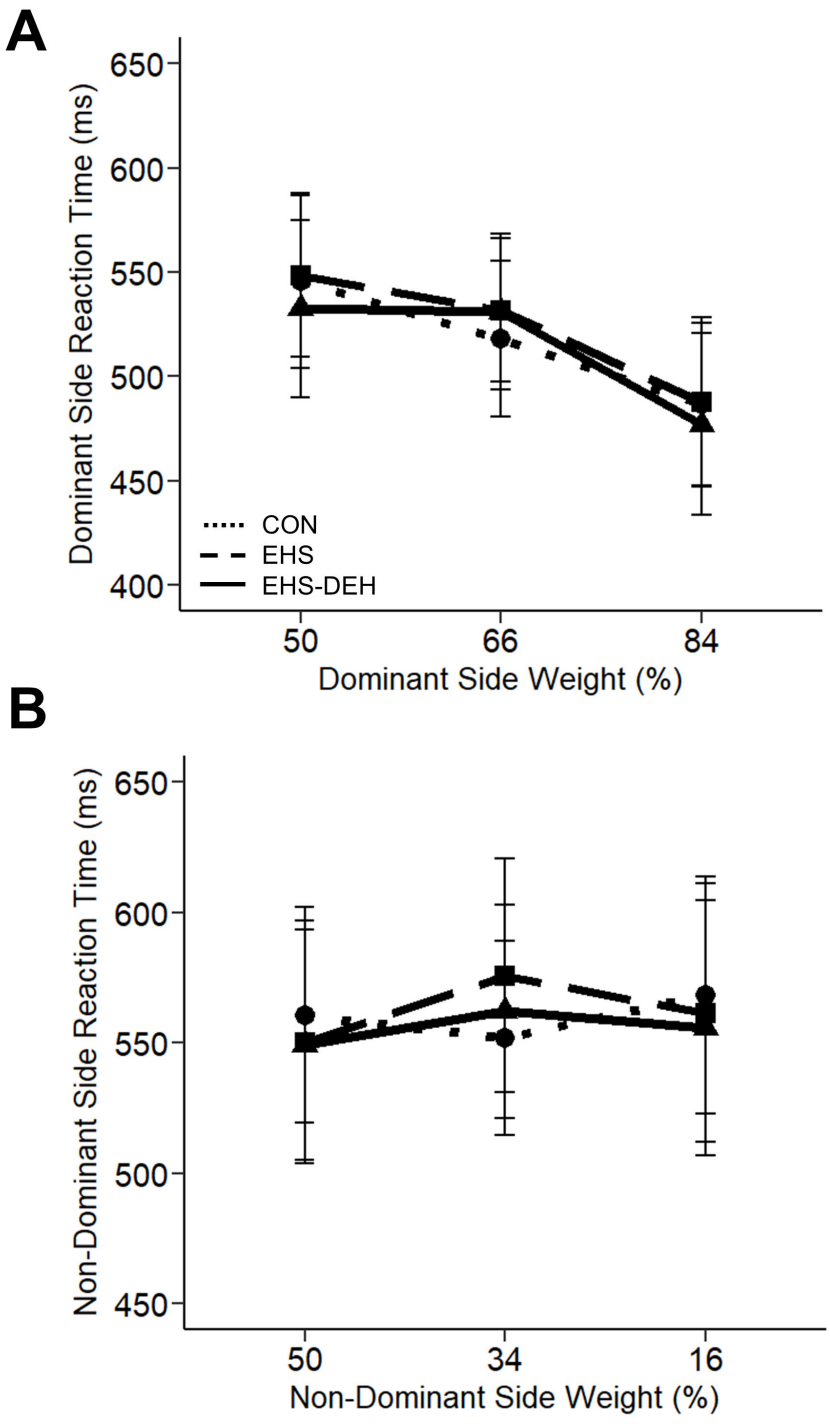


Figure 4.4: Mean ± 95% CI probabilistic choice reaction times for dominant (A) and non-dominant (B) responses following resting control (CON), exercise-heat stress with water replacement (EHS), and exercise heat stress coupled with dehydration (EHS-DEH).

combined was not different among trials (CON: 86.3 $\pm$ 5.5%, EHS: 77.5 $\pm$ 10.0 %, EHS-DEH: 74.1 $\pm$ 11.8%; p = 0.18). Individual data across the three trials is presented in the Appendix (Figure A.2) and reflects variable responses when averaged across all % weightings. There was a significant (p = 0.0004) trial by side dominance interaction. EHS-DEH had no effect on accuracy for the dominant side ($p > 0.05$); however, accuracy was significantly lower (p = 0.04) in the non-dominant side compared to CON but not EHS (p = 0.18). Specifically, this occurred when the non-dominant side constituted $\leq$33% of stimuli (i.e., % dominance side weighting $\geq$66%; $p < 0.05$; Figure 4.5B).

4.4.4 EEG

Figures 4.6-4.9 present the EEG data. Each figure presents: (A) the voltage tracing over time for trials (CON, EHS, and EHS-DEH) to identify when VEP occurred, (B) mean signed area amplitude for dominant and non-dominant side responses averaged across electrodes of interest and time region for all trials, and (C) head maps indicating VEP amplitude averaged across the shaded region in A with the specific electrodes identified. Across all measured VEP components, no significant effects were observed for side dominance ($p > 0.05$) or the side dominance by trial interaction ($p > 0.05$).

The VEP tracings in Figure 4.6A indicate that all trials had a peak activation over the occipital electrodes (O1, OZ, O2) consistent with an N1 (70 - 150 ms). In Figure 4.6B, mean signed area amplitude was significantly different by trial (p = 0.002), as peak amplitude during EHS-DEH (385.2 $\pm$ 141.3 uV*ms) was significantly greater compared to CON (241.8 $\pm$ 168.6 uV*ms; p = 0.001) but not EHS (300.3 $\pm$ 171.1 uV*ms; p = 0.60); CON and EHS were not different (p = 0.60). In Figure 4.6C, this difference is displayed with darker blue shade in the head map over the three occipital electrodes.

No other significant ($p > 0.05$) trial effects were observed across VEP components (Figures 4.7-4.9). Figure 4.7A indicates that all trials had a peak activation over the central electrode (Cz) consistent with the early phase of the CNV (350 - 550 ms after warning

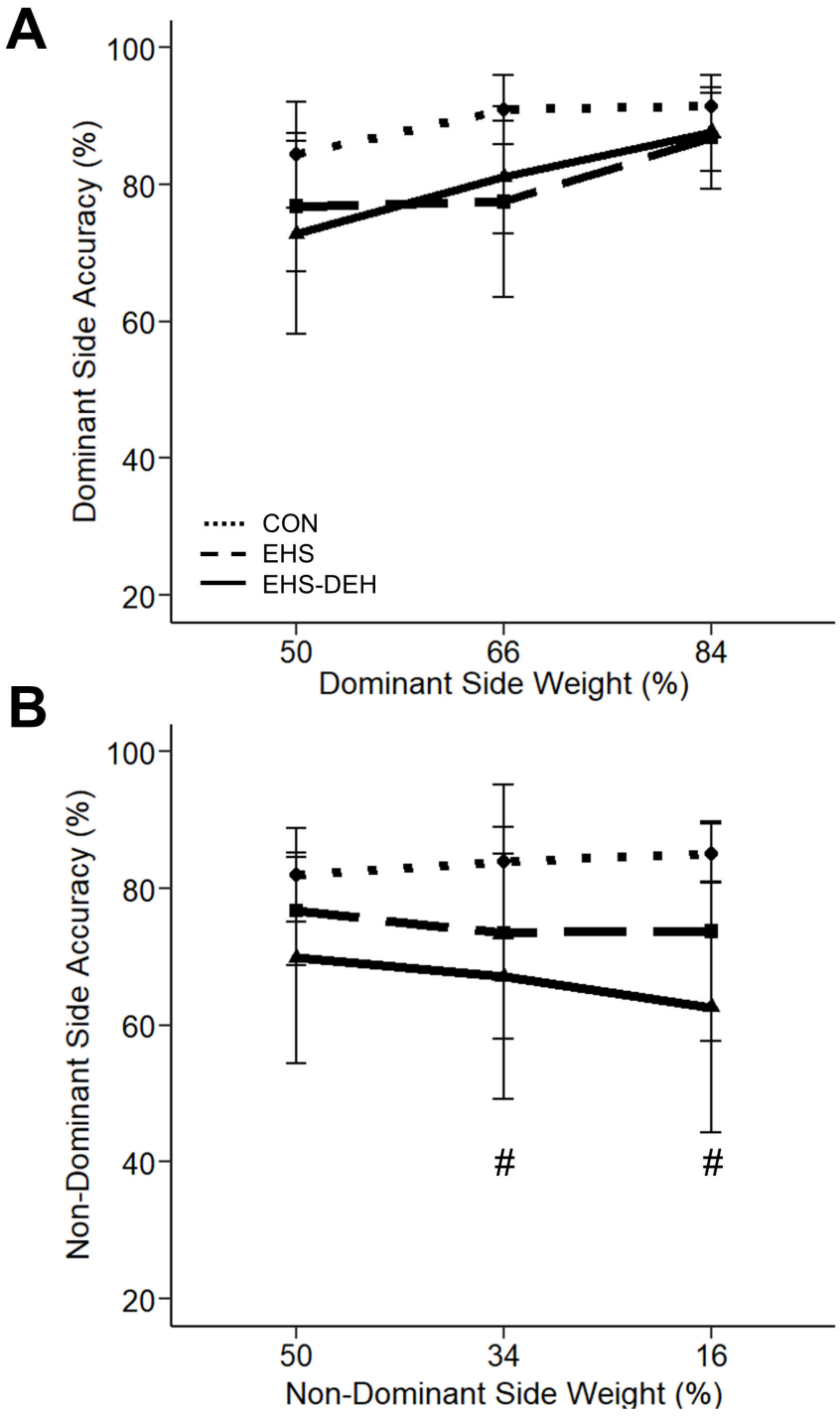


Figure 4.5: Mean $\pm$ 95% CI probabilistic choice reaction time accuracy (%) for dominant (A) and non-dominant (B) responses following resting control (CON), exercise-heat stress with water replacement (EHS), and exercise heat stress coupled with dehydration (EHS-DEH). # indicates significant ($p < 0.05$) difference from CON

Table 4.3: Relationship between visual evoked potentials and probabilistic choice reaction time (PCRT) non-dominant side accuracy and ratings of physical demand during the PCRT.

Visual Evoked Potential	PCRT Non-Dominant Side Accuracy	Rating of Physical Demand
Early CNV	r = 0.13, p = 0.17	r = -0.02, p = 0.46
Late CNV	r = -0.10, p = 0.23	r = 0.19, p = 0.14
N1	**r = -0.34, p = 0.006**	**r = 0.32, p = 0.03**
N2	**r = -0.22, p = 0.04**	r = 0.02, p = 0.44

signal). Mean signed area amplitude was similar (p = 0.49) across trials (CON: 278.62 $\pm$ 107.0 uV*ms, EHS: 259.8 $\pm$ 103.3 uV*ms, EHS-DEH: 319.7 $\pm$ 118.4 uV*ms) as indicated by similar color shading within the highlighted area (Figure 4.7C). Figure 4.8A indicates that all trials had a peak activation over the central electrode (Cz) consistent with a the late phase of the CNV (200 ms prior to movement stimulus). Mean signed area amplitude was similar (p = 0.88) across trials (CON: 161.1 $\pm$ 90.7, EHS: 193.8 $\pm$ 108.2, EHS-DEH: 169.9 $\pm$ 77.4 uV*ms) as indicated by similar color shading within the the highlighted area (Figure 4.8C). Figure 4.9A indicates all trials had a peak activation over the centroparietal electrodes (PZA, C1P, C2P, CZ, C1, C2) consistent with a N2 (180-270 ms). Mean signed area amplitude was similar (p = 0.33) across trials (CON: 278.6 $\pm$ 107.0, EHS: 259.8 $\pm$ 103.3, EHS-DEH: 319.7 $\pm$ 118.38 uV*ms) as indicated by similar color shading within the highlighted area (Figure 4.9C).

Table 4.3 presents the relationship between VEPs and both PCRT non-dominant side accuracy and rating of physical demand from the NASA-TLX. N1 amplitude was significantly (p = 0.006) associated with non-dominant side PCRT accuracy (r = -0.34) and rating of physical demand (r = 0.32, p = 0.03). N2 amplitude was significantly (p = 0.04) associated with non-dominant side PCRT accuracy (r = -0.22, p = 0.04) but not rating of physical demand (p = 0.44). The early and late components of the CNV were not significantly associated with non-dominant side PCRT accuracy or rating of physical demand (p $>$ 0.05).

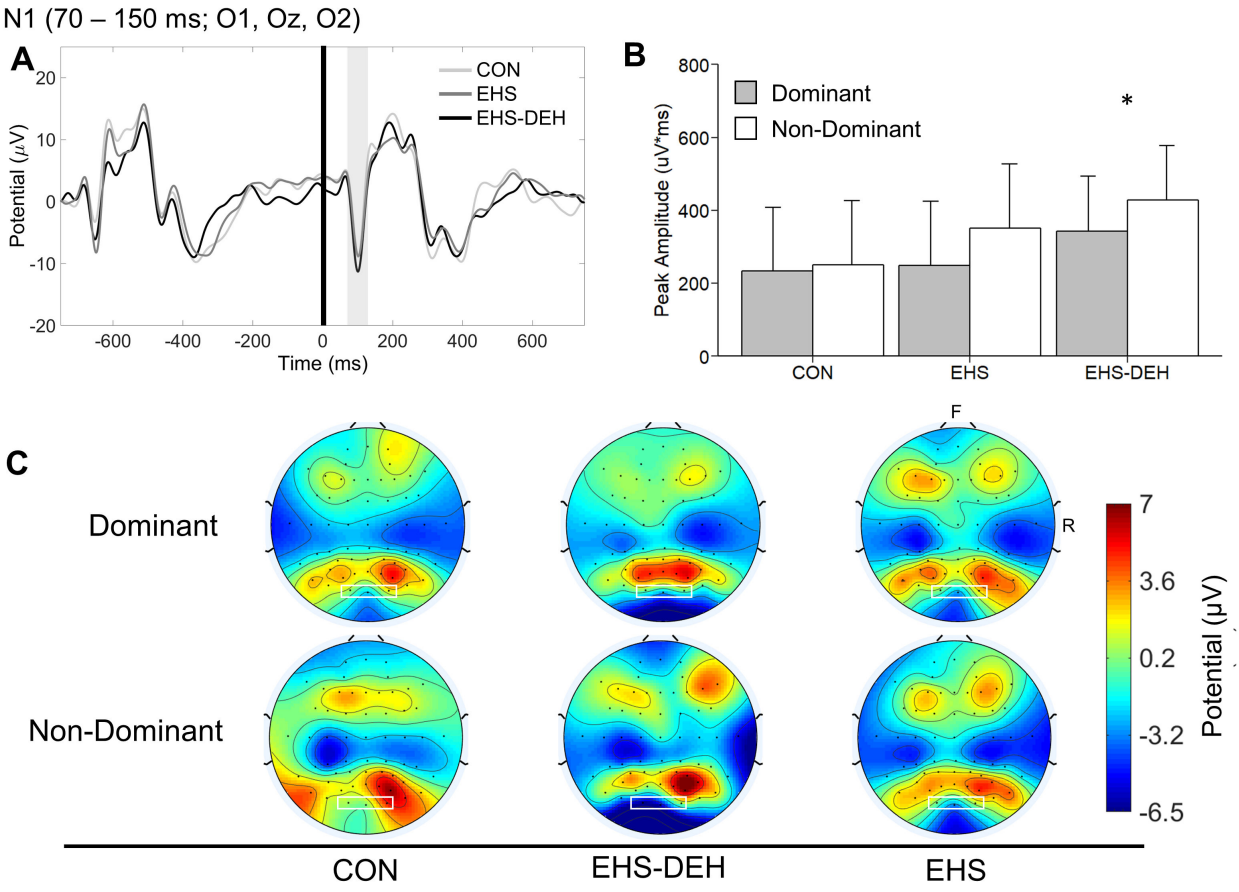


Figure 4.6: *A*: VEP tracing for resting control (CON), exercise-heat stress with fluid replacement (EHS), and exercise-heat stress with dehydration (EHS-DEH) **over occipital electrodes (O1, O2, Oz)** during the probabilistic choice reaction task. Shaded area indicates region of analysis. *B*: Mean ± 95% CI signed peak area amplitude for the N1 component for dominant and non-dominant sides. * significant ($p < 0.05$) trial difference vs. CON. *C*: Head maps of brain activity during the N1 (averaged across shaded area in A) during dominant and non-dominant side responses. Greater N1 amplitude found in EHS-DEH is indicated by darker blue region within the rectangle.

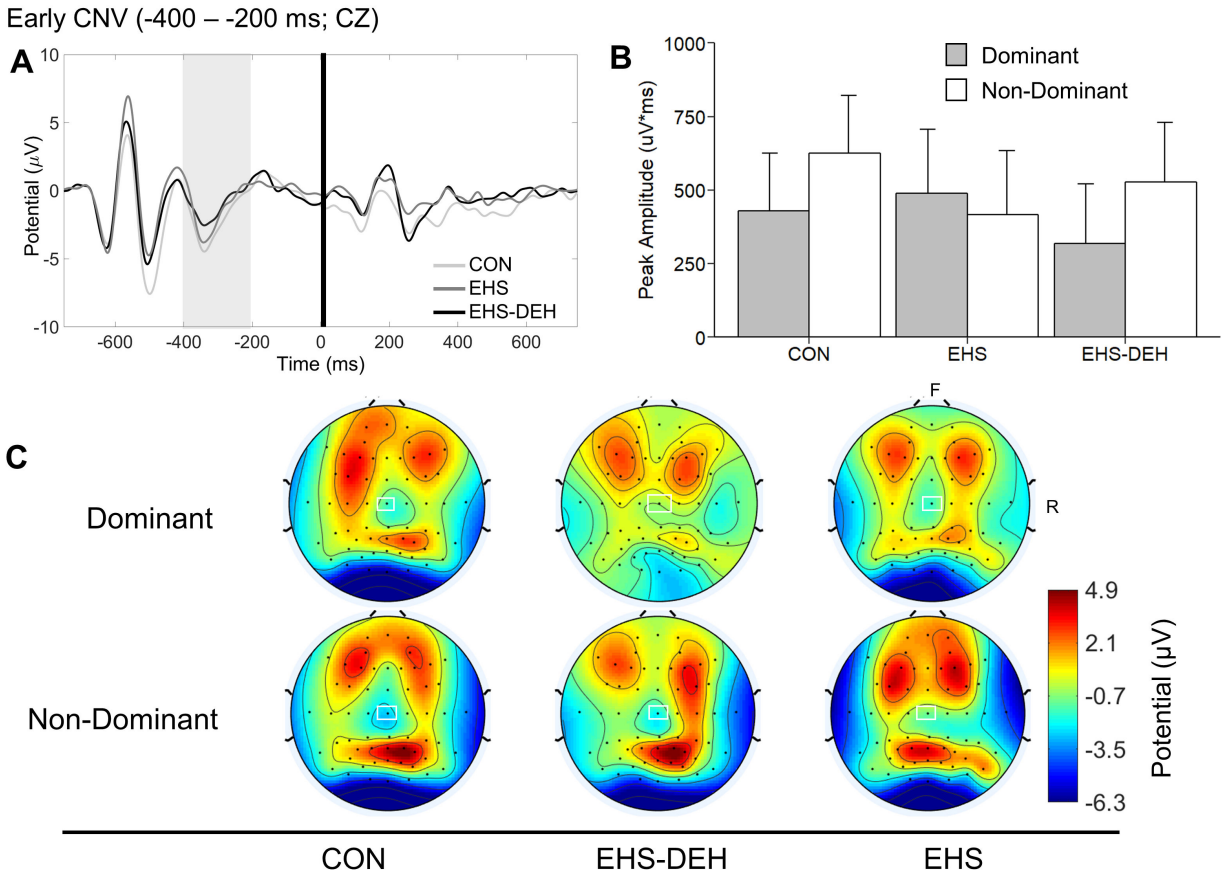


Figure 4.7: *A*: VEP tracking for resting control (CON), exercise-heat stress with fluid replacement (EHS), and exercise-heat stress with dehydration (EHS-DEH) **over the central electrode (Cz)** during the probabilistic choice reaction task. Shaded area indicating region of analysis. *B*: Mean ± 95% CI signed peak area amplitude for the early contingent negative variation (CNV) component for dominant and non-dominant sides. *C*: Head maps of brain activity during the CNV (averaged across shaded area in A) during dominant and non-dominant side responses. No change in amplitude was observed across trials or side dominance as indicated by similar color shading within the rectangle.

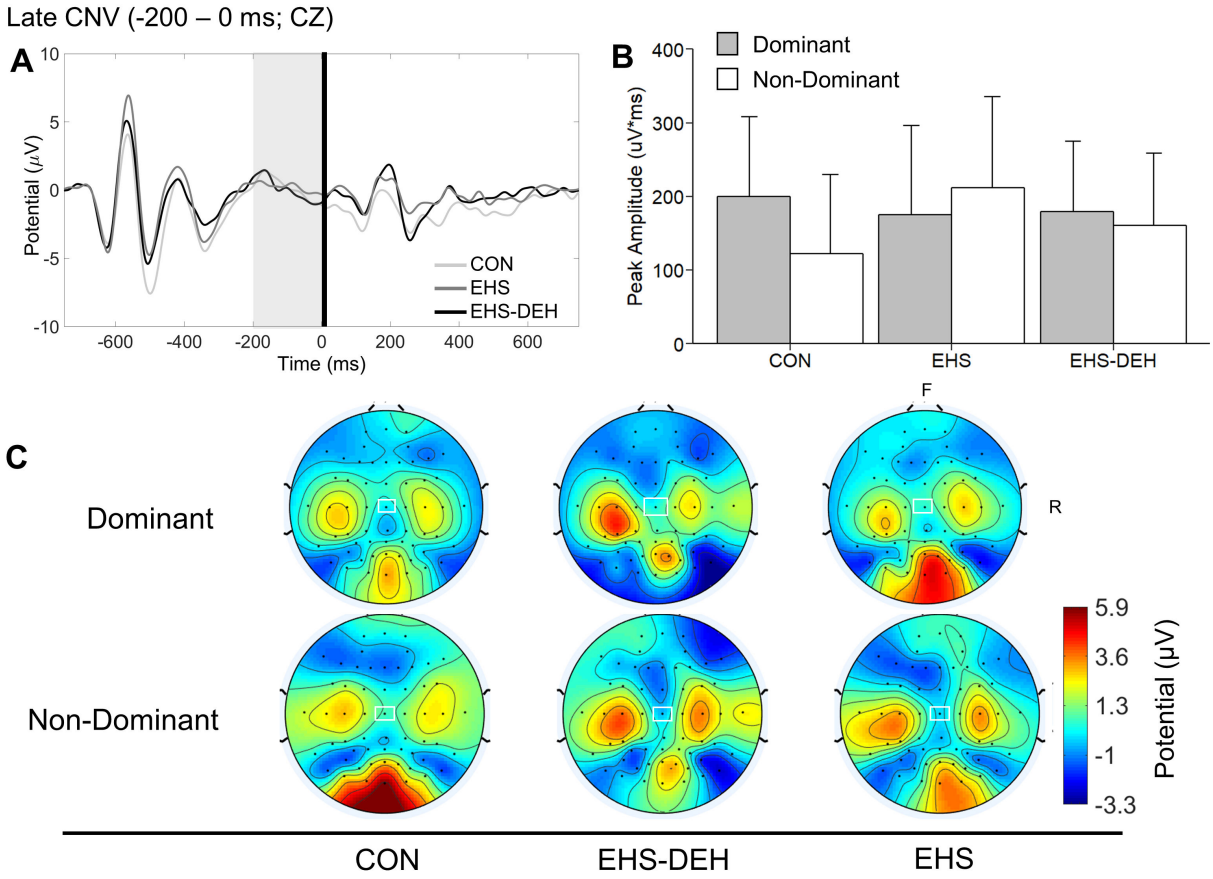


Figure 4.8: *A*: VEP tracking for resting control (CON), exercise-heat stress with fluid replacement (EHS), and exercise-heat stress with dehydration (EHS-DEH) **over the central electrode (Cz)** during the probabilistic choice reaction task. Shaded area indicating region of analysis. *B*: Mean $\pm$ 95% CI signed peak area amplitude for the late contingent negative variation (CNV) component for dominant and non-dominant sides. *C*: Head maps of brain activity during the CNV (averaged across shaded area in A) during dominant and non-dominant side responses. No change in amplitude was observed across trials or side dominance as indicated by similar color shading within the rectangle.

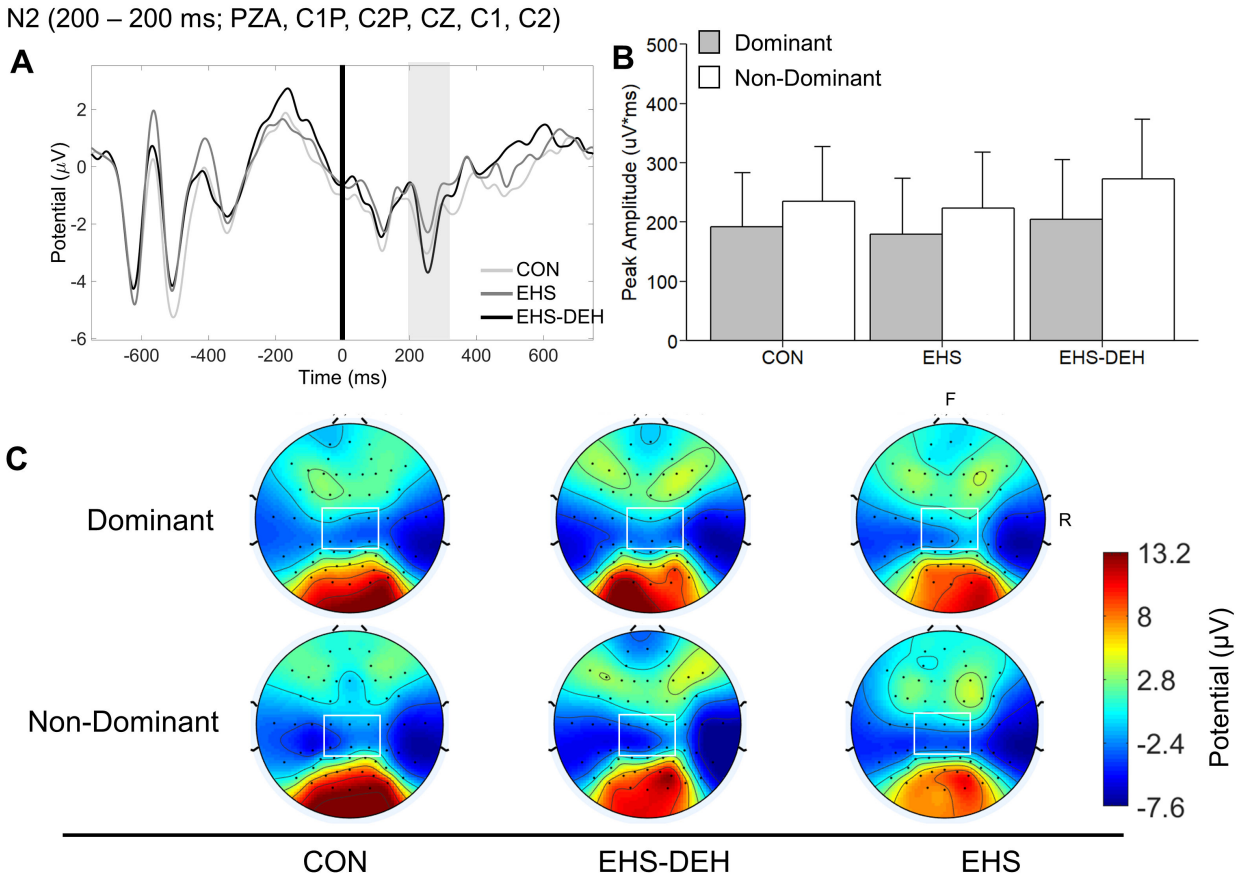


Figure 4.9: *A*: VEP tracking for resting control (CON), exercise-heat stress with fluid replacement (EHS), and exercise-heat stress with dehydration (EHS-DEH) **over centroparietal electrodes (PZA, C1P, C2P, CZ, C1, C2)** during the probabilistic choice reaction task. Shaded area indicating region of analysis. *B*: Mean $\pm$ 95% CI signed peak area amplitude for the N2 component for dominant and non-dominant sides. *C*: Head maps of brain activity during the N2 (averaged across shaded area in A) during dominant and non-dominant side responses. No change in amplitude was observed across trials or side dominance as indicated by similar color shading within the rectangle.

4.5 Discussion

Aim 2 demonstrated that dehydration impairs visuomotor performance, specifically motor execution. One remaining question was whether dehydration degrades performance and brain activity during a task emphasizing another principal component of visuomotor function, motor planning. The PCRT was designed to elevate motor planning demands while maintaining a rhythmic stimulus presentation pattern (i.e., standardizing motor execution). Secondly, EEG was utilized to investigate specific neural processes during visuomotor functioning. By increasing motor planning demands, we can model visuomotor function required for occupational and military tasks [182, 194, 96] such as driving, operating weaponry systems, and piloting aircraft [197].

The first major finding of this study was that prior exercise-heat stress with dehydration impaired performance on a visuomotor task emphasizing motor planning. The PCRT in this study is similar to other choice reaction time tasks (rapid decision-making requiring fine motor movements), although these studies have not uniformly observed impairments with dehydration across a range of BM losses (1-4%) [198, 15, 13, 195, 76]. Our study is the first to use a choice reaction time paradigm to emphasize motor planning, as previous studies only modulated task difficulty by varying the number of possible stimuli from two [195, 76] to five [85]. By manipulating the frequency of stimulus presentation, we attempted to bias the motor planning systems towards the dominant (more frequent) direction [125]. This is consistent with the drift-diffusion model [96], such that less evidence accumulation would be required to move towards the dominant side and more evidence accumulation required for the non-dominant side. Additionally, the longer duration test ($\sim$32 min) was employed to: i) impart different motor planning demands across blocks (as validated in Figure 4.3) and, ii) stress a subcomponent of executive function- attention.

Given the PCRT design, it was not surprising that dehydration elicited performance impairments during the non-dominant stimuli. This was in contrast to dominant side stimuli,

which appeared unaffected by body water deficits. Responding to the non-dominant stimuli imparted greater stress on the motor planning systems [199] and likely required greater attentional control [140]. In accordance with the drift diffusion model of motor planning [96], our data indicate that dehydration likely exacerbated the response bias towards the dominant side. This is potentially explained by one of two findings. First, because motor planning is influenced by previous knowledge [200], dehydration may have elicited errors during recognition of the stimulus pattern frequency. Secondly, humans coordinate movement to minimize effort [201]. In our study, dehydration elicited greater perceived effort compared to resting control during the motor planning task. If, following dehydration, the effort required to respond to the non-dominant stimulus (potentially in conjunction with an exaggerated bias) was too great, individuals may have sacrificed accuracy, resulting in worse performance, as has been observed previously [201, 200]. This finding may also explain why dehydration has not previously impaired choice reaction time performance [13, 198, 15], as the previous assessments have not adequately stressed motor planning function by biasing previous knowledge or creating task demands which would increase effort.

The second important finding of this study was that dehydration increased N1 amplitude compared to control in occipital brain regions. This posterior N1 indicates visual processing demands [121, 202] which may also be related to spatial attention [113]. The occipital N1 is related to visual processing [113] as evidenced by a greater amplitude in subjects who were required to discriminate between two stimuli compared to one [203]. Therefore, I hypothesize that, following dehydration, greater visual processing demands were required. These findings also appear to confirm a hypothesized finding from Aim 2. In Aim 2, increased brain activity within the temporal lobe and hippocampus was observed following dehydration which was believed to indicate greater visual processing demands within the ventral stream [169]. Therefore, the findings in Aim 3 provide additional evidence suggesting the presence of dehydration-mediated increases in visual processing demands. Secondly, occipital N1 amplitude may also be related to spatial attention, specif-

ically attentional processing pertaining to stimulus features (i.e., color, shape) [202, 204]. Thus, these results could also indicate dehydration requires greater effort to direct attention towards the salient feature (color) of the stimulus.

This study is the first to examine multiple stages of neural processing (i.e., sensory and cognitive-motor) during a motor planning task following dehydration. The only previous study to employ a similar approach [85] indicated that P3 amplitude is not affected by dehydration as assessed during an auditory oddball paradigm. My data are consistent in that task-related processing (i.e., stimulus categorization) does not appear to be altered by dehydration. The early and late CNV, measuring processing of the warning stimulus and movement preparation [113], do not appear to be altered with dehydration. N2 amplitude, which represents neural processing relative to stimulus categorization (e.g., frequent, infrequent) [113, 122], was also unaffected by dehydration. Therefore, it is possible that dehydration-mediated performance impairments were a result of visual and/or attentional deficiencies and not to motor planning-specific processing. However, the significant but weak association between N1 amplitude and task accuracy may indicate other aspects of neural processing may be related to performance impairments following dehydration.

Another important finding was that a task emphasizing motor planning elicited greater levels of mental effort (as measured with the NASA-TLX scale) following dehydration. While perceptual measures have often been reported to be adversely impacted by dehydration [76, 80, 74, 75], fewer studies have examined effort of the task itself. One previous study [85] observed greater ratings of effort and concentration during a cognitive battery following 2.6% body mass loss elicited via fluid restriction, but ratings of effort were not relevant to a specific task. Understanding perceptual responses during motor planning is important to occupational-specific tasks (relying on visuomotor function) which may be impaired by dehydration [6, 5]. There was no significant association between Early N1 amplitude and ratings of frustration or effort, indicating the perceived mental workload may not explain changes in motor planning performance.

One theme persisting from Aim 2 is the high levels of individual variability during visuomotor tasks in response to dehydration. We recruited individuals who were all aerobically fit (aerobic fitness $>$ 50 mL/kg/min) in an attempt to control for heat tolerance during the walking trials [20]. However, as in Aim 2, there were several individuals who were able to maintain motor planning performance despite moderate dehydration. Individual variability has not been addressed in previous reviews [72, 128, 86] and, as such, it is unknown whether this persists only in visuomotor tasks (motor planning and execution). One interesting finding is that, following dehydration, some individuals were able to maintain performance despite high levels of effort and frustration.

This study also conflicts with finding from Aim 2 that exercise-heat stress alone impaired visuomotor performance and, when exercise-heat stress was coupled with dehydration, further impairments were observed. The reasons for this discrepancy are not entirely clear. While we did observed a similar pattern of accuracy decrements during non-dominant responses under exercise-heat stress, no significant differences were observed. One possible rationale for these divergent findings is that exercise-heat stress impairs motor execution more than motor planning, although this has not been previously examined. Secondly, if the subject population in the current study was able to tolerate the exercise heat-stress better (more aerobically fit) [20], this could also explain why resting control and exercise-heat stress with water replacement had similar PCRT findings. This study also differs from Aim 2 in that brain activations were not different between exercise-heat stress and exercise-heat stress with dehydration. While there appeared to be a similar trend to PCRT accuracy, no significant differences were found.

4.6 Conclusion

In summary, this study expands upon prior efforts to demonstrate that motor planning is impaired by dehydration. In combination with Aim 2, these data identified that both components of visuomotor function, motor execution and motor planning, are impacted by

body water deficits. This study made the following novel observations: 1) performance on a task emphasizing motor planning is impaired with exercise-heat stress coupled with dehydration compared to resting control conditions; 2) motor planning impairments following dehydration may result from greater resources required for visual processing and spatial attention; and 3) perceived mental workload during a task requiring motor planning was elevated following dehydration. These observations indicate dehydration may impair visuomotor performance, specifically for tasks requiring sustained repetitive movements and vigilance which are key elements for ensuring occupational safety.

CHAPTER 5
SUMMARY AND FUTURE DIRECTIONS

5.1 Summary of Dissertation Findings

Dehydration is a common physiological stress encountered in many occupational, military, and athletic environments. While the physical performance impairments elicited by dehydration are well described [17, 16, 18], much less is known about the effects on cognitive-motor performance. Furthermore, previous research indicates occupational (e.g., pilot simulation) and athletic motor-skill tasks (e.g., basketball and golf skills) also degrade with dehydration [103, 6, 104]. However, it is unclear whether these decrements may result, in part, from cognitive-motor impairments (e.g., inability to judge distance to target or recognize and make corrective actions). This dissertation attempted to first understand if and how dehydration may impair cognitive-motor performance (e.g., task domain, level of body water losses) and then investigate one element common to both cognitive-motor tasks and occupational/athletic tasks: the visuomotor system.

Previous studies examining the effect of dehydration on cognitive-motor performance have largely been equivocal [86, 72]. The reasons for the disparate literature are many and might be explained by the several factors differing across experiments including methods to elicit dehydration, the specific cognitive-motor tests employed, and the magnitude of dehydration [72, 86, 73]. This dissertation initially assessed the impact of design factors using a systematic review coupled with meta-analysis, followed next by two experimental protocols performed at body water deficits deemed sufficient to elicit physiological compensatory responses [50] and using comparison conditions to independently assess the effect of the method (prior exercise-heat stress) from dehydration.

In Aim 1, a systematic review of the literature and meta-analysis indicated that de-

hydration imparts a small, but significant impairment on cognitive-motor performance. Furthermore, higher order cognitive functions (i.e., executive functions broadly defined as including attention, working memory, cognitive flexibility) [140, 134] were at greater risk for impairments compared to 'lower level' domains (e.g., simple reaction time; Table 5.1). I also observed the magnitude of dehydration was associated with the effect size (e.g., severity), potentially indicating a dose-response relationship between body water losses and cognitive-motor deficit. Data from Aim 1 also indicated that dehydration $>2\%$ body mass loss may be the "threshold" level for decline, as cognitive-motor impairments were more pronounced compared to $\leq 2\%$.

Another observation from Aim 1 identified a gap in the literature, specifically, that the effects of dehydration on the visuomotor system (performance and neural activity) were insufficiently studied. Additionally, brain structures may be altered by dehydration [46, 5, 49], although reports were inconsistent and incomplete. Aim 2 indicated that visuomotor performance, specifically motor execution during a finger tapping task, was impaired by prior exercise-heat stress with additional impairments occurring when prior exercise-heat stress was coupled with dehydration. Dehydration also increased brain activation within motor areas during the finger tapping task which did not occur with exercise-heat stress alone. Secondly, Aim 2 demonstrated that dehydration expands the brain ventricles along with reduced volume of adjacent brain tissue structures (e.g, thalamus, cerebellum) but the opposite structural changes occurred when water consumption matched sweat loss during exercise-heat stress. Changes in the ventricular and periventricular structure volumes were inversely related to plasma osmolality changes (e.g., hypertonicity in dehydration and hypotonicity when sweat is replaced with electrolyte-free water) but were not associated with finger tapping accuracy. Aim 2 therefore indicates that dehydration elicits impairments to a core visuomotor function occurring concomitant with increased brain activations but could not be linked as a consequence of brain structural changes.

A remaining question from Aim 2 was whether dehydration impacts motor execution

or the integrated neural processing needed prior to movement initiation (i.e., motor planning). To address this, I designed a task in Aim 3 which maintained the same rhythmic structure as the finger tapping task in Aim 2 but with additional motor planning demands through utilizing bimanual responses and differential weighting of stimuli between hands. Secondly, specific neural processes via EEG and VEPs were investigated to assess if dehydration alters both perceptual and cognitive processing of stimuli. Dehydration increased the mental effort and decline in accuracy during a motor planning task although only during non-dominant (less frequent) stimuli. Dehydration also resulted in an increased N1, indicating increased visual processing and spatial attention demands.

Table 5.1 presents a summary of the comprehensive findings relevant to brain structure, brain function, and cognitive/visuo-motor performance. Overall, this dissertation identified that dehydration impairs visuomotor accuracy as evidenced by impairments during the motor planning and execution tasks. Interestingly, greater decrements were observed during motor execution compared to a task which also included elevated visuomotor planning demands (PCRT). The explanation for this finding is not entirely clear. One potential reason is the pacing differences between the VMPT (1 Hz) and PCRT (0.66 Hz). There is a strong relationship between task performance, timing frequency, and sequence complexity [93]. It is possible, then, that stimulus frequency may elicit greater impairments following dehydration than "higher" levels of visuomotor processing. Future studies should manipulate this parameter, specifically through increasing paced stimuli presentation rate to $>$3Hz, as this frequency appears be a threshold for significantly increased error rates under resting conditions [93]. Another common thread through all aims of this dissertation is the contribution of attentional capacity to cognitive-motor decline following dehydration. This is a meaningful observation, as the ability to stay vigilant during monotonous tasks is a large contributor to workplace incidents and driving accidents [205].

Our data also indicates that dehydration alters brain activation patterns during visuomotor processing. The BOLD responses indicated, similar to other studies [46], that dehy-

Table 5.1: Summary Effects of Prior Exercise-Heat Stress (EHS) with and without dehydration on brain anatomy, visuomotor function, and brain activity using electroencephalography (EEG)or functional magnetic resonance imaging (fMRI). All results are compared to resting control. ND = No difference. Superscript number denotes aim responsible for findings.

	Prior EHS - No Dehydration	Prior EHS with Dehydration
Brain Anatomy		
Total Brain Volume[2]	ND	ND
Cortical Brain Structures[2]	ND	ND
Ventricular System[2]	Contraction	Expansion
Subcortical/Periventricular Tissues[2]	Expansion	Contraction
Brain Activation During:		
Motor Execution[2]	ND	Increased in Motor and Visual Areas (fMRI)
Motor Planning[3]	ND	Increased Visual and Spatial Attention Demands (EEG)
Visuomotor Function		
Motor Execution[1]	Accuracy Impaired (8%)	Accuracy Impaired (16%)
Motor Planning[3]	ND	Non-Dominant Side Accuracy Impaired (16%)
Mental Effort[3]	ND	Greater
Cognitive-Motor Performance		*All Methods (Including EHS)*
Executive Functions[1]		Impaired
Information Processing[1]		ND
Memory[1]		Impaired
Reaction Time[1]		ND

dration increases brain activation in task specific and other areas (i.e., temporal lobe and hippocampus). However, the block design implemented resulted in only being able to examine brain activity as averaged during a 30 s block. During the motor planning task (Aim 3), neural processing relevant to stimulus onset were examined, indicating that dehydration increased visual processing and spatial attention demands. Furthermore, one commonality observed within this dissertation were changes in visual processing following dehydration within the ventral visual stream (Aim 2) and occipital lobe (Aim 3). Thus, brain function (activity) requires more effort following dehydration, specifically within the visual system. However, I cannot disregard potential of competing demands between the task, physiological monitoring (i.e., thirst as monitored by the anterior cingulate), and other extraneous factors (i.e., worsened well-being). The presence of thirst and greater mental workload may contribute to the increased visual and/or attentional demands following dehydration which, potentially, contribute to performance decrements.

5.2 Alternative Hypotheses and Future Directions

Figure 5.1 illustrates the hypothesized model for these series of experiments. Water deficits (via sweat loss) or hypertonic hypovolemia increases plasma osmolality which in turn would mirror CSF osmolality eliciting osmotic fluid shifts out of brain tissue (thalamus and cerebellum) into the adjacent ventricular spaces. These changes in brain structure would theoretically lead to increased functional demands (greater BOLD) in areas involved with motor function and thus ultimately impair cognitive motor function. This model appears to hold true for explaining the changes in brain structure; but, these structural changes did not correlate with the diminished accuracy in Aim 2. However, since altered brain function (increased BOLD during Aim 2 and decreased attentional gating from Aim 3) did occur in parallel with impair visuomotor accuracy, alternative explanations need to be explored to elucidate the mechanisms responsible for dehydration-mediated impaired cognitive-motor performance.

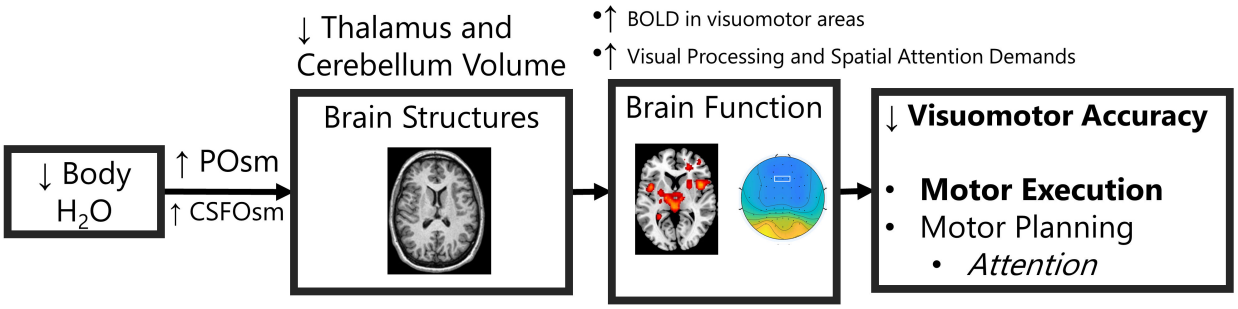


Figure 5.1: Integrated model of how dehydration may impair visuomotor performance. POsm = Plasma osmolality, CSFOsm = cerebrospinal fluid osmolality, BOLD = Blood Oxygen Level Dependent Response.

The results of this dissertation have not fully determined whether changes in brain structures following exercise-heat stress (with and without water replacement) alter cognitive-motor performance. While I did not observe associations between changes to brain structures and visuomotor performance, it is still unclear whether a countermeasure which maintains brain tissue and ventricular volume prevents cognitive-motor impairments. Numerous studies have identified that quenching thirst and correcting fluid losses are effective in preserving/improving cognitive-motor performance [9, 10, 146, 206, 207] although a recent meta-analysis [208] was not conclusive in the support of these findings. Furthermore, these studies did not assess brain morphology; it is therefore unknown whether changes in brain structures were present when cognitive-motor performance was preserved with water replacement. Therefore, the first proposed study would follow the protocols in Aim 2 and 3, except using a fluid countermeasure isotonic to sweat losses which would mitigate plasma dilution and, theoretically, not alter brain morphology. If similar cognitive-motor performance impairments to Aim 2 are observed following isotonic fluid replacement, this would indicate another mechanism underlies these degradations.

One such mechanism not directly examined in my dissertation was the effect of stress and central fatigue elicited by the exercise-heat stress used to elicit dehydration. Exercise in warm environments produces a stress response as evidenced by increased concentrations of prolactin and norepinehrine in the plasma [209, 84]. Furthermore, dehydration also in-

dependently elicits a stress response by increasing serotonin and catecholamine turnover [210, 211, 212]. The presence of stress increases neural activity (and catecholamine depletion), specifically within areas responsible for stress (locus coeruleus, hypothalamus, hippocampus) [213, 214], which can impair cognitive-motor performance [215, 216, 217]. Thus, an alternative hypothesis for my integrative model is that visuomotor performance was impaired primarily via a graded (and possibly additive) stress response elicited by exercise-heat stress alone and exacerbated when exercise-heat stress was coupled with dehydration. Therefore, it is possible cognitive-motor impairments following dehydration do not result from body water deficits per se, but instead the physiological compensation (i.e., increased sympathetic response) required for homeostasis.

Future studies to test this alternative hypothesis could employ dehydration methods with multiple (exercise-heat stress plus dehydration) versus a single stressor (fluid restriction, diuretic). While Aim 1 results suggest fluid restriction elicits similar cognitive-motor impairments as exercise-heat stress, 24-h fluid restriction (-2.6% BM) has been previously demonstrated to not impair brain function or performance [85] in healthy adults. To date, no study has compared dehydration elicited via fluid restriction and exercise-heat stress on brain structure, function, and cognitive-motor performance. Currently, it is also unclear whether isotonic dehydration impairs cognitive-motor performance; the Aim 1 systematic review returned only two studies employing diuretics to elicit marginal body water deficits [80, 76]. Isotonic dehydration will elicit compensatory physiological responses (renal water retention and peripheral vasoconstriction) [18, 218, 37] without osmotic gradients and, in accordance with Aim 2, may not result in brain tissue volume (e.g., thalamus) changes. Therefore, by examining different methods of dehydration (specifically those at rest and without exercise-heat stress), a better understanding of mechanisms underpinning dehydration-mediated cognitive-motor impairments (i.e., stress and brain structural changes) can be gained.

If cognitive-motor impairments following dehydration result from central catecholamine

depletion related to the stress response, the precursor to catecholamine synthesis, tyrosine, could be an effective countermeasure [216, 211]. Tyrosine has been previously shown to reverse cognitive-motor impairments resulting from cold water immersion [216], suggesting that up-regulating catecholamine synthesis alleviates stress-induced cognitive-motor impairments. Thus, potential future studies could be similar to Aim 2 except adding: i) the provision of tyrosine before exercise-heat stress (but still utilizing water replacement) or ii) a small tyrosine bolus throughout exercise-heat stress (with and without full fluid replacement). These studies, therefore, may indicate that cognitive-motor performance can be preserved despite stress elicited by exercise in the heat with and without fluid replacement, suggesting performance decrements are a result of central catecholamine depletion.

Another future study employing sleep deprivation as a comparative stressor could further elucidate the effects of dehydration-mediated stress response on brain function and performance. Sleep deprivation is known to elicit large cognitive-motor performance impairments [129, 219, 220] through a neurophysiological stress mechanism (i.e., increased neurotransmitter turnover) [221, 222]. Like dehydration, sleep deprivation appears to effect executive functions to a greater extent than other cognitive-motor domains [129] along with similar effects on brain function [223] (e.g., increased activity within the thalamus) to maintain performance. Therefore, a future study might compare, in a stepwise fashion, the cognitive-motor responses of dehydration, sleep deprivation, and sleep deprivation with dehydration. It is possible that sleep deprivation combined with dehydration would elicit exacerbated cognitive-motor impairments (specifically within executive function domains) compared to sleep deprivation and dehydration alone. Furthermore, evidence of an additive stress response would be indicated by elevated brain activity, specifically within areas previously identified to be involved with dehydration and sleep deprivation: the thalamus and hippocampus. Observing additive brain function and performance decrements with a known stressor (and subsequent decreases in central catecholamine levels) would provide strong evidence of the stress response being the underlying mechanism behind dehydration-

mediated cognitive-motor impairments.

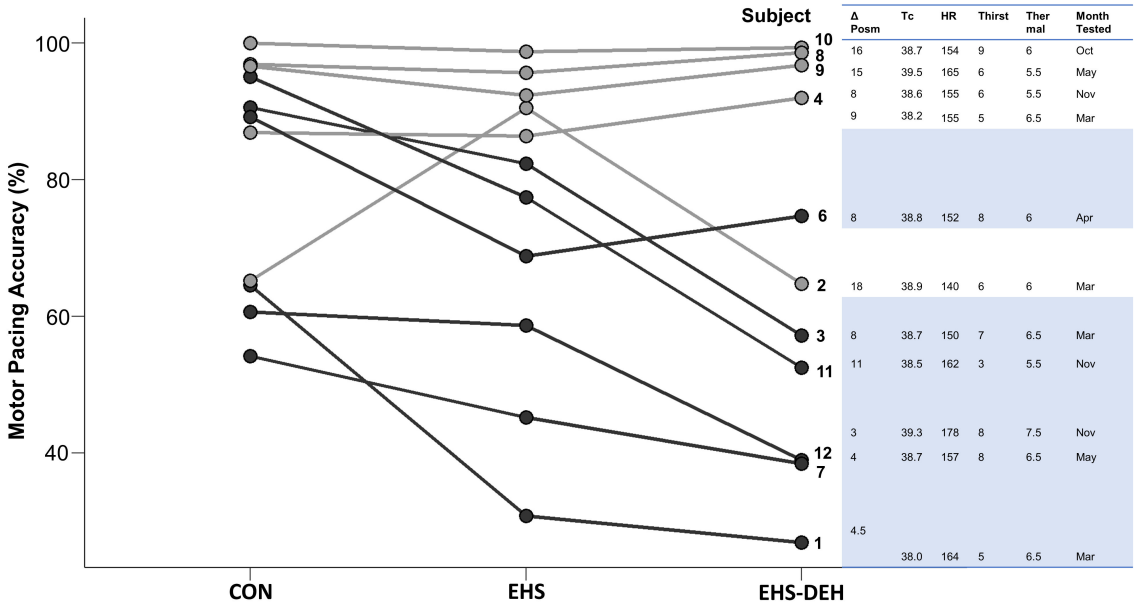
5.2.1 Future Directions

Individual Variability in Cognitive-Motor Responses

One remarkable observation from Aim 2 and 3 was that some individuals appear to have greater risk for visuomotor deficits following dehydration. While data from Aim 2 indicates a significant association between brain structures and plasma osmolality (POsm), this did not predict impairment in visuomotor performance as there was large intra-individual variation and inconsistency. Moreover, an alternative interpretation could be that some individuals (for currently unknown reasons) are more prone to large effects of dehydration while many others are resistant. This would explain the inconsistency among study findings in the literature and also suggest that, although a "small" effect size is observed on average, the effect size, in reality, is either large or trivial among individuals.

An attempt was made to screen for potential susceptibility. When examining individual biomarkers (Figure 5.2) such as core temperature, heart rate, thirst rating, thermal sensation, heat acclimation status (based solely on month of the year tested), and change in plasma osmolality, no one variable appeared to stand out linking susceptibility to dehydration. One potential variable is sweat sodium concentration, which is highly variable among individuals [224]. Those with high sweat sodium concentrations ($>$ 90 mmol/L) may be more reflective of an isotonic hypovolemia (less change in plasma osmolality but greater plasma volume decrease) with dehydration [225]. Secondly, those who have elevated sweat sodium losses that fully replace (100% sweat loss) with plain water are potentially at greater risk for swelling of the periventricular tissues. Recently, regional sweat sodium concentrations from the forearm or thigh have been shown to be accurate with regards to whole body sweat rate [224] and therefore this could be implemented in subsequent studies.

Another potential explanation for individual risk is that those with the highest accuracy tended to maintain it- indicating a screening tool might be developed to identify at risk indi-



Δ Posm	Tc	HR	Thirst	Thermal	Month Tested
16	38.7	154	9	6	Oct
15	39.5	165	6	5.5	May
8	38.6	155	6	5.5	Nov
9	38.2	155	5	6.5	Mar
8	38.8	152	8	6	Apr
18	38.9	140	6	6	Mar
8	38.7	150	7	6.5	Mar
11	38.5	162	3	5.5	Nov
3	39.3	178	8	7.5	Nov
4	38.7	157	8	6.5	May
4.5	38.0	164	5	6.5	Mar

Figure 5.2: Aim 2 motor pacing accuracy (% correct responses) across resting control (CON), exercise-heat stress with water replacement (EHS), and exercise-heat stress with no water replacement (EHS-DEH). Table to right demonstrates potential biomarkers for performance during EHS-DEH such as change in plasma osmolality (POsm), core temperature (Tc) and heart rate (HR) at end of walking, thirst rating, thermal sensation rating, and month tested.

viduals. Those individuals with higher VMPT proficiency (i.e., >90% accuracy that did not diminish over time in control) appeared unaffected by dehydration or prior exercise-heat stress without dehydration. While some exceptions are present (e.g., subject eleven in Figure 5.2 who had high accuracy during resting control), it could be that those with a higher cognitive reserve persevered. Individuals with greater cognitive reserve [226] will exhibit a greater maximal cognitive capacity and therefore can tolerate adverse physiological conditions without visuomotor decrements. This occurred in a somewhat large proportion of subjects (four out of thirteen), but the reasons are unclear. In much of the previous literature (and in this dissertation), the subject population mostly consists of college students with presumably large cognitive reserves [226].

Furthermore, individuals who appear resilient to the effects of dehydration may have a greater ability for sustained attention. We acknowledged the VMPT is characteristic of

a task stressing the attentional capacity of the individual. Because the VMPT is a simple visuomotor task, it is possible that subjects had low levels of arousal, leading to a state of mental underload [227]. Mental underload is a mental workload which falls below the optimum range and can elicit detrimental performance on par with mental overload [227]. Thus, it is possible that those with greater attentional capacity may be able to increase effort without performance impairments. This is also salient given data from Aim 1 indicating that dehydration significantly impairs attentional capacity. Therefore, it may be valuable to have baseline measures of attentional capacity to use as a predictor of responses to dehydration.

5.2.2 Older Adults

While the current thesis (and the literature at large) investigated adults (age: 18 - 45), more attention directed into populations with greater susceptibility to dehydration. Older adults ($>$ 65 y) are at greater risk for dehydration given several factors outlined previously [228, 229, 230]: i) decreased concentration capacity of the kidney, ii) decreased efficacy of arginine vasopressin receptors in the kidney, iii) lower total body water due to reduced muscle mass (by $\sim$8%), iv) a muted thirst response to both hypovolemia and hypertonicity, v) potential for diminished water intake if non-ambulatory, vi) prescription medications (e.g., loop diuretics) which alter water balance, and vii) taste preferences in beverages. In combination with the increased risk of dehydration, older adults are also at greater risk of presenting with acute confusion [231]. Acute confusion, a mental condition consisting of increased irritability, distracted thinking, and memory impairments, has severe functional implications [231, 232, 3]. Previous research has suggested a link between euhydration and prevention of acute confusion [3, 233], but the extent to which dehydration contributes is unclear.

Another factor to consider in studying older adults is age-related changes in ventricular volume. Ventricle expansion along with crenation of other brain structures were ob-

served in an aged population [153], suggesting older adults (defined as $>$ 60 y) may have a pre-existing pattern of neuroanatomical changes, analogous to what we observed with dehydration. It would be of interest to determine whether dehydration imparts a further stress exacerbating pre-existing ventricular expansion/periventricular tissue shrinkage and impairs visuomotor performance in tasks mirroring processes involved in daily living (e.g., reaching, grasping). Because older adults are already at risk for visuomotor impairments [234], it is important to understand the potential influence of dehydration, specifically in relation to tasks observed to be at risk with body water deficits (e.g., driving) [44].

Based upon our meta-analysis, most studies are on young adults (18-35y), indicating that older adults ($>$ 65 y) are a severely underrepresented population in such studies. This is an important omission given the well-understood cognitive decline [235] in this population. While our review of literature was primarily aimed at studies on adults (not youth), only one additional study was found using older adults [236]. This study utilized older adults undergoing bowel preparation for surgery, which decreased body mass and total body water by 2.0 and 2.6%, respectively [236]. No significant differences were observed in tests of executive function (trail making test) following dehydration. However, this study was not a within-subjects design (test-retest), alternatively utilizing cross sectional comparison between other subjects undergoing a similar surgery without bowel preparation [236]. Furthermore, the act of bowel preparation likely elicits an isotonic hypovolemia rather than a hypertonic hypovolemia used in the present study.

5.2.3 Additional Neuroimaging Approaches

I believe an area of future work should involve novel neuroimaging approaches to examine both resting neural activity and connectivity patterns following dehydration. For example, the brain function-performance impairments during sleep deprivation occur, in part, from an inability to "shut off" resting state and "turn on" task-specific neural networks [129]. This known function-performance relationship, therefore, could be leveraged to compared

Network	Network description
1	Default mode network: bilateral inferior parietal lobule, posterior cingulate, bilateral superior frontal gyrus, and medial frontal gyrus
2	Dorsal attention network: bilateral intraparietal sulcus, precentral and superior frontal sulci, ventral precentral, and middle frontal gyrus
3	Posterior visual processing network: retinotopic occipital cortex and temporal-occipital regions
4	Auditory-phonological: bilateral superior temporal cortex
5	Precentral, postcentral, and medial frontal gyri, primary sensory-motor cortices, and supplementary motor area
6	Self Referential Activity: medial-ventral prefrontal cortex, anterior cingulate, hypothalamus, cerebellum

Table 5.2: Table of resting state networks as described previously [237]

whether dehydration impairs cognitive-motor performance in a similar fashion. Previous research with both functional magnetic resonance imaging (fMRI) and electroencephalography (EEG) has identified six resting state networks (Table 5.2) spread throughout the brain with specific functional implications. Specific to the current dissertation, thalamocortical activity was noted in networks 1, 3, 4, and 5 (Table 5.2) [237], and therefore these networks could be proved as evidence for the effect of dehydration on visuomotor function. Previous research has identified a positive relationship between resting default mode network activity and working memory performance, suggesting resting state neural activity may influence cognitive performance [238]. Previous exercise-heat stress may also alter resting state networks, as indicated with modified resting state activity within the cingulate, precuneus, and visual cortex following hyperthermia [239]. I would hypothesize that increased resting brain activity would be observed following sleep deprivation and dehydration, indicating a similar stress-response relationship.

While our data suggest dehydration increases brain activation required to complete a visuomotor task, the genesis of that increased neural resource requirement remains unclear. One potential analytical technique to investigate this question is connectivity analysis with dynamic causal modeling (DCM; Fig 5.3). DCM allows for comparison between different models of connectivity during cognitive-motor tasks [240, 241]. Given that I observed

greater bilateral fMRI activation with dehydration, altered neural connectivity could explain these findings. DCM analysis has previously expanded the understanding of human behavior during finger tapping (at 0.75 Hz) by identifying the existence of top-down cognitive control to facilitate task performance [242].

DCM could also help understand how dehydration impacts visuomotor functioning by further examining how different aspects of task design (e.g., task demands, interval duration, neurotransmitter levels) [151, 243] and physiological status impact connectivity patterns. A previous review observed that, during predictable motor tasks with a short duration and length of inter-stimulus interval, activations likely occur within the bilateral supplementary motor area, left primary motor cortex, and left primary somatosensory cortex [243]. Cognitive-motor timing ($>$ 1 s inter-stimulus duration) additionally requires activation of higher brain areas, such as the bilateral prefrontal cortex [243]. Based upon previous research [242], DCM analysis with the seed regions of: left primary motor cortex, bilateral supplementary motor area, bilateral thalamus, visual cortex, and bilateral prefrontal cortex could reveal how these areas are integrated to accomplish visuomotor function. With this analysis, I would hypothesize that dehydration would manifest in greater generative model probability (i.e., bidirectional connectivity, Figure 5.3b-c) within the automatic timing circuit but also integration of higher brain areas (prefrontal cortex).

5.2.4 Occupational Relevance

Previous research has indicated that sport-specific motor skills (e.g., golf, basketball) [103, 104] and occupation specific task performance (e.g., driving, pilot simulation) [5, 6] are impaired following dehydration. Occupational environments predisposing individuals to heat strain (and therefore dehydration) may also pose a risk [7], but specific decrements relating to visuomotor performance have not been reported. It is also typical in such environments to wear protective clothing, which can both accelerate sweat loss [**bishop˙limitations˙nodate**] and increase heat strain [244], leading to a greater risk and magnitude of dehydration. Ther-

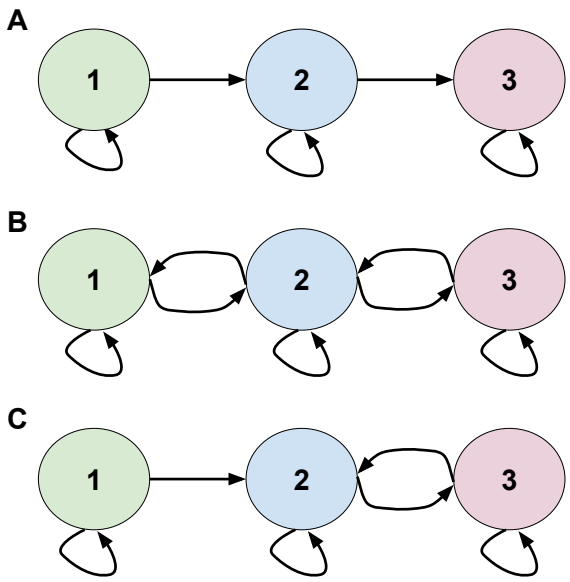


Figure 5.3: Potential Dynamic Causal Modeling Results. Arrows indicate direction of connectivity between hypothetical brain regions (1-3). Unidirectional arrows indicate one-way flow of information whereas bidirectional arrows indicate reciprocal connections between two specific brain regions. Arrows connecting to the same brain area represent self-inhibitory activation.

mal stress without changes in hydration status (i.e., core temperatures $>39\ ^{o}$C) is believed to impair cognitive-motor performance [245, 246] with visuomotor possibly being more susceptible [246]. Furthermore, this may be particularly problematic given that it is common for individuals to begin their work shift dehydrated [247, 248]. Therefore, effects of dehydration on visuomotor function during thermal stress has particular relevance to safety and occupational performance.

Dehydration can also affect occupations not associated with adverse environmental conditions. Prolonged dehydration (i.e., beginning and ending shift with insufficient body water) has been observed across a variety of occupations, including those requiring high-level cognitive functioning [248]. Within a hospital setting (indoors, adequate water availability) 36% of nurses and doctors began their work shift dehydrated (urine osmolality $>$ 800 mOsmol/kg) and the portion of individuals dehydrated increased to 45% at the end of the shift [248]. Dehydration was also related to short-term memory deteriorations [248],

which, given the high cognitive load of specific occupations, could have further catastrophic patient safety implications.

5.3 Conclusions

This dissertation examined the effects of dehydration on the neural systems which govern our visually-guided movements, the visuomotor system. The observations on visuomotor function were that dehydration: i) elicits a significant graded impairment to cognitive-motor performance, more pronounced in higher versus lower-order functions, ii) elicits plasma hypertonicity associated with brain structural changes (fluid shifts out of periventricular tissue into adjacent ventricle spaces), iii) degrades visuomotor performance during both the motor planning and motor execution phases, and iv) increases brain activations within the motor and sensory areas during visuomotor tasks. In contrast, avoiding dehydration during exercise in the heat through water consumption also did not prevent structural changes in the brain (although these occurred in an opposite direction with plasma osmolality dilution) or maintain visuomotor performance (e.g. motor execution phase only). Thus, changes in brain structure did not explain impairments in the accuracy of performing repetitive fine motor tasks. Further investigations are needed to elucidate the mechanism(s) contributing to task impairments following dehydration coupled with exercise-heat stress and why some individuals are resilient to cognitive-motor decline. Specifically, future studies should determine if the present findings are consistent with other forms of dehydration (e.g., isotonic hypovolemia) and induction methods (e.g., without heat stress) and the magnitude of effect when combined with other stressors (e.g., sleep deprivation). Other potential areas of further investigation are the effects of dehydration on populations across a range of ages (youth, elderly) and psychological health status, susceptibility/resilience in cognitive-motor performance, and the translational application to important occupational tasks.

Appendices

APPENDIX A

SUPPLEMENTAL FIGURES AND TABLES

Table A.1: Characteristics of the 32 studies examining the effects of dehydration (DEH) on cognitive performance. RF = Recreationally Fit, HF = Highly Fit (VO2peak: $>$ 55 mL/kg/min) , NR = Fitness Not Reported, EHS = Exercise-Heat Stress (Ambient Temperature $\geq 27^{o}$C), FR = Fluid Restriction, BM = Body Mass, M = Males, F = Females. ND = No difference between DEH and control conditions. All studies were repeated measures design. Italicized cognitive domains were include in executive functions.

Reference	Subjects / Fitness Status	BM Loss (%)	DEH Method	Control Condition	Cognitive Tests	Cognitive Functions	Reported Effects of DEH ($p < 0.05$)
Armstrong et al. [80]	25 F / RF	1.4	EHS, EHS + Diuretic	Exercise + Fluids	Four Choice Reaction Time	Reaction Time	ND
					Psychomotor Vigilance Test	Reaction Time	ND
					Matching to Sample	*Working Memory*	ND
					Grammatical Reasoning	*Executive Function*	ND
					Scanning Visual Vigilance	*Attention*	Increased False Alarms
					Repeated Acquisition	Memory	ND
Baker et al. [148]	11 M / HF	1,2,3,4 Reported at Mean = 2.5%	EHS	EHS + Fluids	Test of Variables of Attention	*Attention*	Decreased Vigilance
Barroso et al. [83]	12 M / HF	1.8	EHS	Rest	Simple Reaction Time	Reaction Time	Improved Reaction Time
Bijlani et al. [68]	14 M / NR	3	EHS	Rest	Choice Reaction Time	Reaction Time	ND
					Proof Reading Test	Executive Function	ND
Choma et al. [147]	14 M / HF	6.2	FR	Rest	Digit Span, Story Recall	*Working Memory*	Decreased Recall
Cian et al. [74]	8 M / HF	2.8	Heat, Exercise	Exercise + Fluids	Picture Recall	Memory	Shorter String Recall
					4-Choice Serial Reaction Time	Reaction Time	ND
					Perceptive Discrimination	Information Processing	Increased Reaction Time
					Digit Span	*Working Memory*	Reduced String Length
					Unstable Tracking	Motor Coordination	Greater Deviation
Cian et al. [75]	7 M / HF	2.6	Heat, Exercise	Exercise + Fluids	Picture Recall	Memory	ND
					Choice Reaction Time	Reaction Time	ND
					Perceptive Discrimination	Information Processing	Longer Reaction Time
					Digit Span	*Working Memory*	Shorter String Recall Length
					Unstable Tracking	Motor Coordination	ND

Table A.1 Continued

Reference	Subjects / Fitness Status	BM Loss (%)	DEH Method	Control Condition	Cognitive Tests	Cognitive Functions	Reported Effects of DEH ($p < 0.05$)
D'Anci et al. [15]a	16 M, 14 F / HF	1.8	Exercise	Exercise + Fluids	Digit Span	*Working Memory*	ND
					Simple, Choice Reaction Time	Reaction Time	ND
D'Anci et al. [15]b	12 M, 12 F / HF	1.2	Exercise	Exercise + Fluids	Map Recall	Memory	ND
Ely et al. [13]	32 M / NR	4.0 4.2	EHS	EHS + Fluids	Psychomotor Vigilance Task	Reaction Time	ND
					4-Choice Reaction Time	Reaction Time	ND
					Matching to Sample	*Working Memory*	ND
					Grammatical Reasoning	*Executive Function*	ND
Epstein et al. [244]	9 M / NR	2.4	Heat	Rest	Target Evaluation and Shooting	Information Processing	Impaired Accuracy Greater Errors
Faerevik et al. [249]	8 M / NR	1.5	Heat	Rest	Vigilance Test	Attention	Increased Incorrect Reactions
					Vienna Determination United Test	*Executive Function*	ND
Ganio et al. [76]	26 M / RF	1.6	Exercise	Exercise + Fluids	Four Choice Reaction Time	Reaction Time	ND
			Exercise + Diuretic		Psychomotor Vigilance Test	Reaction Time	ND
					Matching to Sample	*Working Memory*	ND
					Grammatical Reasoning	*Executive Function*	ND
					Scanning Visual Vigilance	*Attention*	Increased False Alarms
					Repeated Acquisition	Memory	ND
Gopinathan et al. [71]	11 M / RF	1,2,3,4	EHS	EHS + Fluids	Serial Addition, Trail-Marking Test	*Executive Function*	Decrease Correct (Addition) Reduced Performance (Trail)
					Word Recognition	Short Term Memory	Less Correct Responses
Grego et al. [141]	8 M / HF	3.1	Exercise	Rest	Map Recognition	*Executive Function*	Impaired Accuracy Faster Reaction Time
					Critical Flicker Fusion Test	Information Processing	Decreased Perception

Table A.1 Continued

Reference	Subjects / Fitness Status	BM Loss (%)	DEH Method	Control Condition	Cognitive Tests	Cognitive Functions	Reported Effects of DEH ($p < 0.05$)
Kakos (2013) [81]	11 M / RF	2.6	EHS	Rest	Running Memory Cont. Performance	*Attention*	ND
					Logical Relations	*Executive Function*	ND
McGregor et al. (1999) [250]	9 M / HF	1.3, 2.4	Exercise	Rest	Mental Concentration Test	Information Processing	ND
McMorris et al. [84]	8 M / RF	2.8	EHS	Rest	Random Movement Generation	*Working Memory*	Worse Test Score
					Choice Reaction Time	Reaction Time	ND
					Corsi Block Tapping	Short Term Memory	ND
Morley et al. [251]	10 M / HF	1.6	EHS	Rest	Psychomotor Vigilance Task	Reaction Time	ND
					Repeatable Episodic Memory Task	Short Term Memory	ND
Patel et al. [105]	24 M / NR	2.5	FR + Exercise	Rest	Simple reaction Time	Reaction Time	ND
					Math Processing,	*Executive Function*	ND
					Standardized Assessment of Concussion		ND
					Match to Sample Task,	*Working Memory*	Impaired Accuracy
					Sternberg Memory Task		ND
Pruna et al. [252]	12 M / HF	2.4	Exercise	Exercise + Fluids	Visuomotor Training Device	Reaction Time	ND
					Serial Sevens Test	*Executive Function*	ND
Serwah et al. [195]	8 M / RF	1.7	EHS	EHS + Fluids	Choice Reaction Time	Reaction Time	ND
Sharma et al. [70]	8 M / RF	1, 2, 3	EHS	EHS + Fluids	Psychomotor test	Motor Coordination	Decreased Score at $\geq$ -2% BM
					Substitution Test	Information Processing	Fewer Correct at $\geq$ -2% BM
					Concentration Test	*Working Memory*	Fewer Correct at $\geq$ -2% BM
Smith et al. [104]	7 M / RF	1.5	FR	Rest	Golf-Specific Cognitive Ability	*Executive Function*	Impaired Distance Judgment
Tomporowski et al. [77]	11 M / HF	1.3, 2.3, 3.7	EHS	Rest	Executive-Processing Task	*Executive Function*	Improved Switch Costs
							Increased Switch Trial Errors
					Brown-Peterson Test	Short Term Memory	ND

Table A.1 Continued

Reference	Subjects / Fitness Status	BM Loss (%)	DEH Method	Control Condition	Cognitive Tests	Cognitive Functions	Reported Effects of DEH ($p < 0.05$)
Turner el al. [82]	11 F / RF	1.5	EHS	EHS + Fluids	Detection Task	Reaction Time	ND
					Choice Reaction Task		ND
					Groton Maze Chase Test	Motor Coordination	ND
					One Card Learning Task	Short Term Memory	ND
					One & Two Back Task	*Working Memory*	ND
					Associate Learning		ND
					Groton Maze Learning Test	*Executive Function*	ND
					Set Shifting Test		ND
van den Heuval [127]	8 M / NR	3,5	Heat	Rest	N-Back Test	*Working Memory*	ND
					Visual Perception Task	Information Processing	ND
Watson et al. [5]	11 M / NR	1.1	FR	Rest	Monotonous Driving Task	*Attention*	Greater Errors after 30 min
Weber et al. [135]	32 M / HF	2.4, 4.8	FR, FR + Exercise	Rest	Simple Reaction Time	Reaction Time	ND
					Executive Function		ND
Wilson et al. [253]	8 M / RF	1.8	Exercise + Sweat Suit	Exercise	Simple Reaction Time	Reaction Time	ND
					Go-No-Go Task	*Executive Function*	ND
Wittbrodt et al. [79]	12 M / RF	1.5	EHS	EHS + Fluids	Letter-Digit Substitution,	Information Processing	ND
					Pattern Comparison		ND
					Perceptual Vigilance	*Attention*	ND
					Trail Making Test	*Executive Function*	ND
					Match-to-Sample	*Working Memory*	ND
Wong et al. [102]	10 M, 9 F / RF	1.4 - 2.1	EHS	Rest	Detection Task	Motor Coordination	Decreased Speed
					Identification Task	*Attention*	Decreased Performance
					Visual Leaning Task,	*Working Memory*	Increased Speed and Decreased Accuracy in Males
					Working Memory Task		ND
					List Based Task	Short Term Memory	Decreased Accuracy

Table A.2: Reaction times (in ms) during the Probabilistic Choice Reaction Time (PCRT) Task following resting control (CON), exercise-heat stress with fluid replacement (EHS), and exercise-heat stress with dehydration (EHS-DEH). Columns indicate percentage of dominant stimuli in task block (n = 300 stimuli).

	PCRT Block (% Dominant Stimuli)		
	50%	**66%**	**84%**
Dominant Side Responses			
CON	545.3 ± 40.7	518.1 ± 34.3	486.5 ± 39.8
EHS	548.2 ± 37.0	532.3 ± 33.1	487.6 ± 40.0
EHS-DEH	529.8 ± 38.1	528.8 ± 34.1	477.0 ± 41.8
Nondominant Side Responses			
CON	560.7 ± 44.0	551.1 ± 38.2	568.3 ± 43.1
EHS	550.1 ± 45.0	575.9 ± 41.8	561.5 ± 47.4
EHS-DEH	546.1 ± 43.3	558.9 ± 39.7	556.7 ± 46.5

Table A.3: Accuracy (% correct) during the Probabilistic Choice Reaction Time task (PCRT) following resting control (CON), exercise-heat stress with fluid replacement (EHS), and exercise-heat stress with dehydration (EHS-DEH). Columns indicate percentage of dominant stimuli during the task block (n = 300 stimuli).

	PCRT Block (% Dominant Stimuli)		
	50%	**66%**	**84%**
Dominant Side Responses			
CON	84.3 ± 8.4	90.9 ± 8.9	91.4 ± 5.6
EHS	76.8 ± 9.2	77.5 ± 13.2	86.8 ± 10.2
EHS-DEH	73.3 ± 12.1	81.8 ± 10.5	87.7 ± 9.2
Non-Dominant Side Responses			
CON	82.0 ± 7.2	83.9 ± 8.0	85.1 ± 5.8
EHS	76.7 ± 9.2	73.5 ± 14.3	73.7 ± 12.2
EHS-DEH	71.0 ± 16.1	68.4 ± 14.4	62.6 ± 13.4

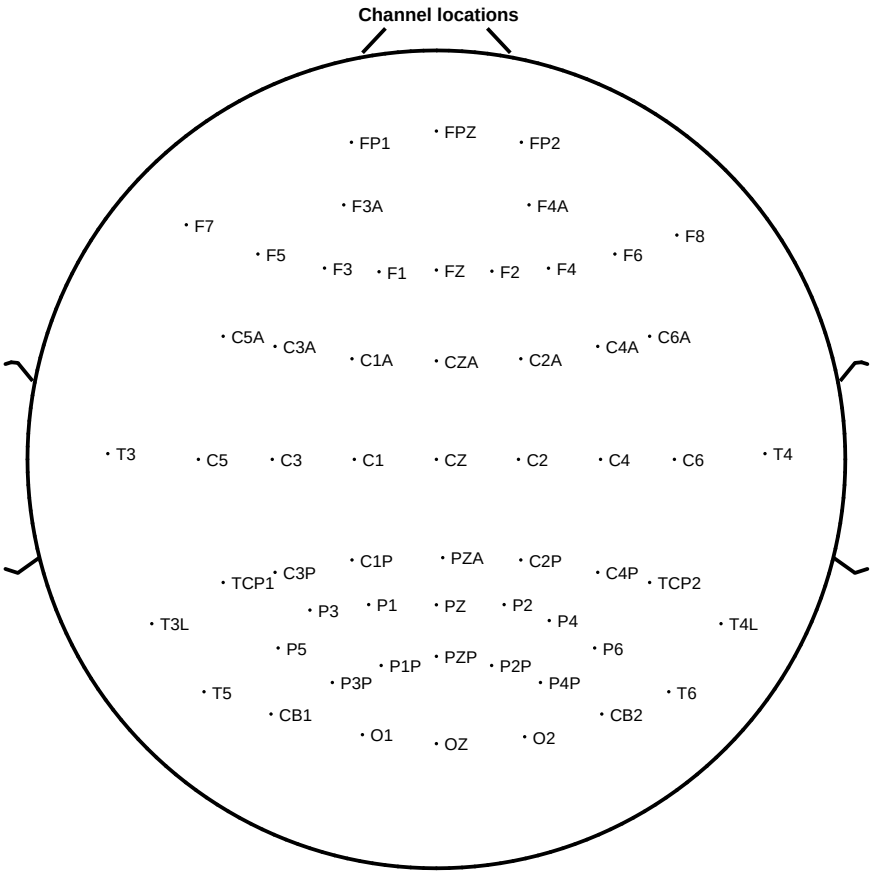


Figure A.1: International 10-20 System Electrode Placement for EEG

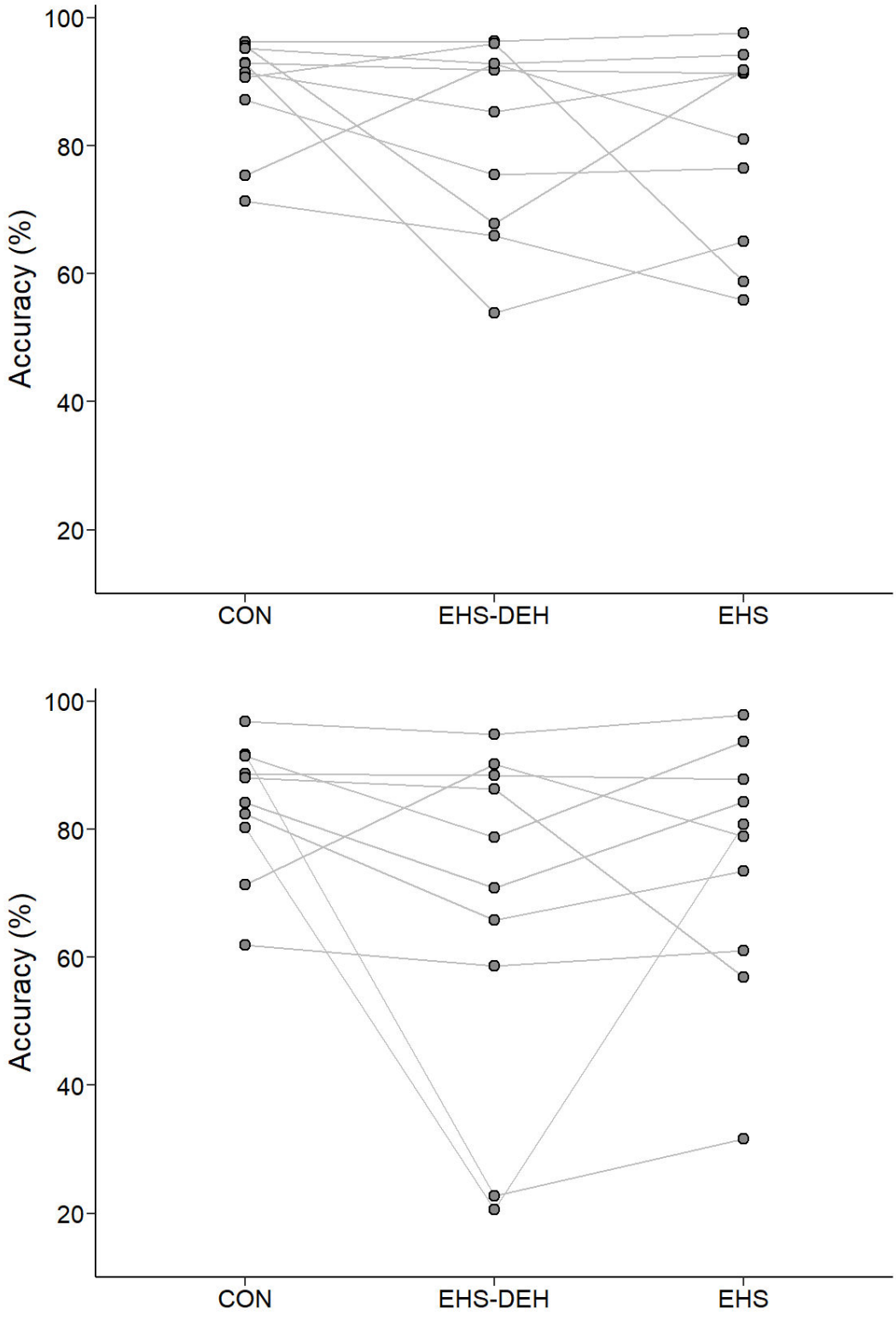


Figure A.2: Individual responses (circles) for dominant (top) and non-dominant (bottom) responses during the probabilistic choice reaction time task following resting control (CON), exercise heat stress with water replacement (EHS), and exercise heat stress coupled with dehydration (EHS-DEH).

APPENDIX B

SURVEY INSTRUMENTS AND QUESTIONNAIRES

Figure 8.6

NASA Task Load Index

Hart and Staveland's NASA Task Load Index (TLX) method assesses work load on five 7-point scales. Increments of high, medium and low estimates for each point result in 21 gradations on the scales.

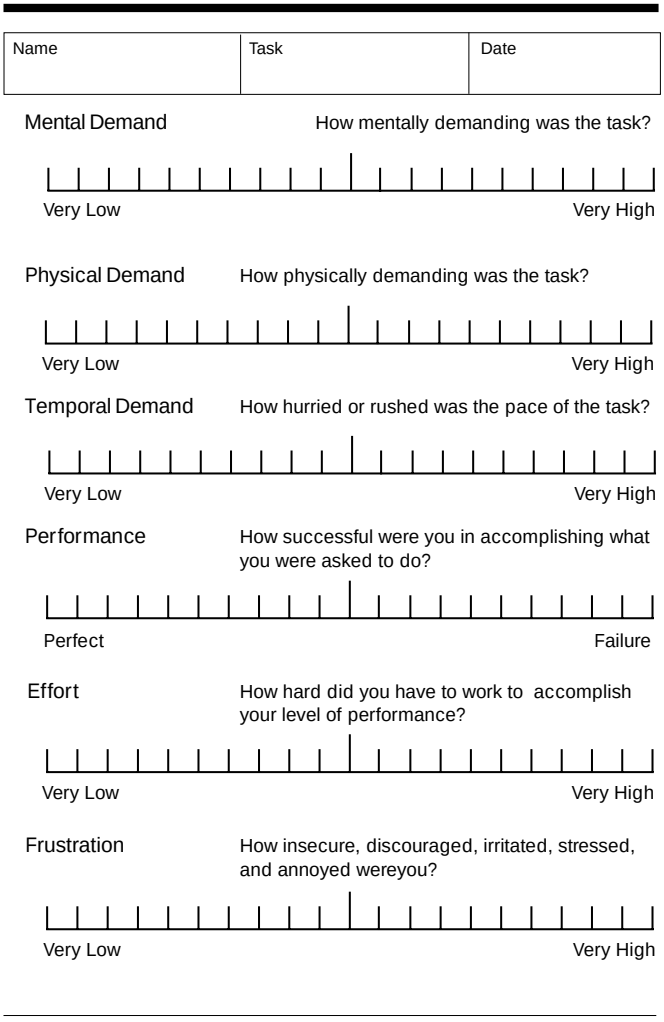


Figure B.1: NASA-TLX Scale [188]

BORG SCALE OF PERCEIVED EXERTION

6	
7	**Very, Very Light**
8	
9	**Very Light**
10	
11	**Fairly Light**
12	
13	**Somewhat Hard**
14	
15	**Hard**
16	
17	**Very Hard**
18	
19	**Very, Very Hard**
20	

Figure B.2: Borg Rating of Perceived Exertion [187]

Gagge Thermal Sensation Scale

0.0	Unbearably Cold
0.5	
1.0	Very Cold
1.5	
2.0	Cold
2.5	
3.0	Cool
3.5	
4.0	Neutral (Comfortable)
4.5	
5.0	Warm
5.5	
6.0	Hot
6.5	
7.0	Very Hot
7.5	
8.0	Unbearably Hot

Figure B.3: Gagge Thermal Sensation Scale [254]

Figure B.4: Thirst Scale

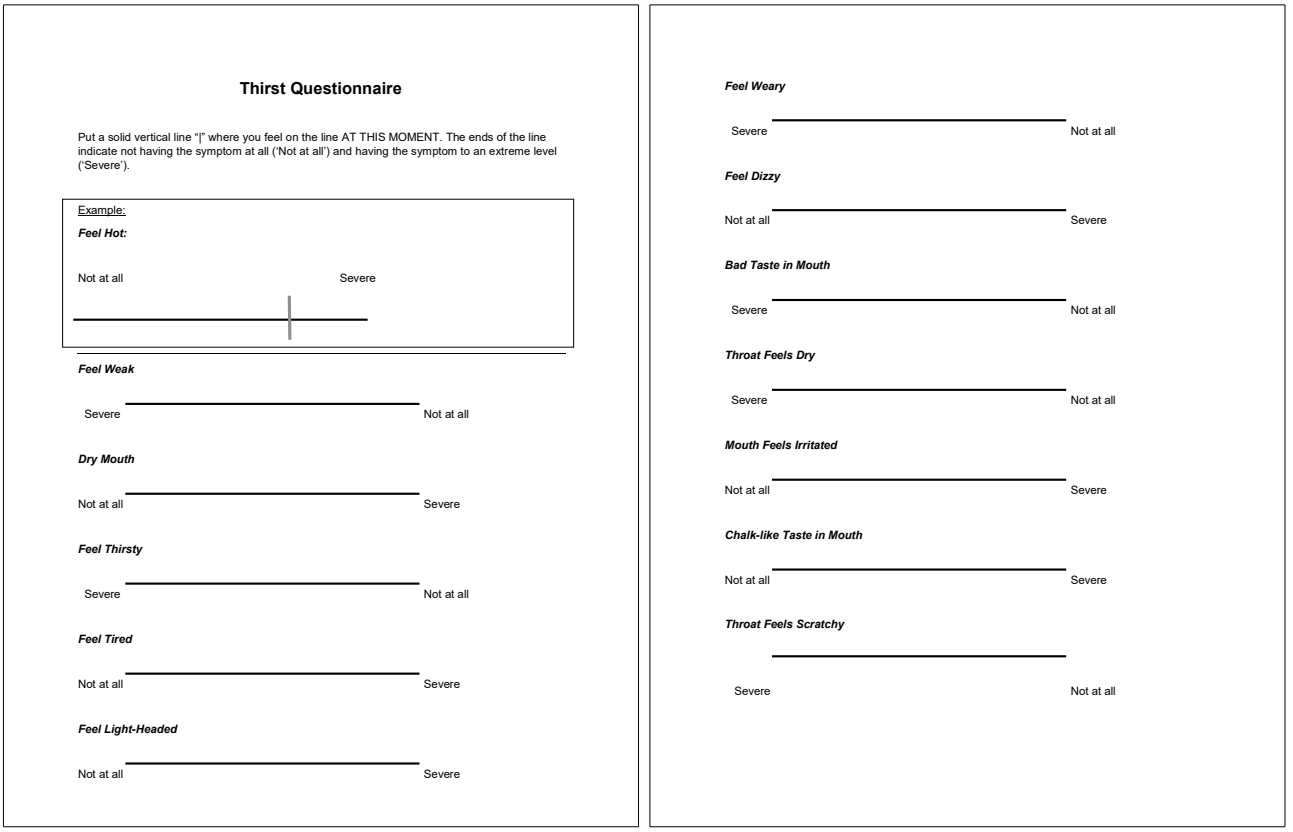


Figure B.5: 10 cm visual analog scale for ratings of thirst. Modeled after [101].

Initial Session: VO2max and DEXA Protocol

Subject Number: _____ **Date:** _____

Sign DEXA Consent Form

DEXA

Height: _____ **Weight:** _____

Room Temperature: _____ Room Humidity: _____

Subjects are told: *One of the purposes of this test is to determine your maximal exercise intensity. Because of that, we need you to give us your best effort. The exercise test continues until you cannot exercise any longer. When you cannot go any further, place both feet to the side of the belt and we will slow the treadmill down so you can cool-down."*

MET cart start time: _____

Time	Speed	Gradient	RPE	HR	VO2	Notes
0-2		0				
2-4		2.5%				
4-6		5%				
6-8		7.5%				
8-10		7.5%				
10-12		7.5%				
12-14		7.5%				
14-16		7.5%				
16-18		7.5%				
18-20		7.5%				

MET cart end time: _____ ; **VO2max:** _____

Figure B.6: Data Collection Sheet for Graded Exercise Test

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